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(54) Title: ISOPRENOID PRODUCTION

(57) Abstract: The invention provides methods and materials related to the production of isoprenoids. Specifically, the invention provides isolated nucleic acids, substantially pure polypeptides, host cells, and methods and materials for producing various isoprenoid compounds.

ISOPRENOID PRODUCTION

BACKGROUND

1. Technical Field

5 The invention relates to methods and materials involved in the production of isoprenoids.

2. Background Information

Isoprenoids are compounds that have at least one five-carbon isoprenoid unit.
10 Examples of isoprenoid compounds include, without limitation, carotenoids, isoprenes, sterols, terpenes, and ubiquinones. Various enzymatic pathways in plants, animals, and microorganisms result in the synthesis of isoprenoid compounds. Typically, isopentenyl diphosphate (IPP), dimethylallyl diphosphate (DMAPP), or combinations thereof are polymerized to form isoprenoid compounds.

15 Two pathways can be used to produce IPP. The first pathway, known as the mevalonate-dependent pathway, produces IPP from 3-hydroxymethyl-3-methylglutaryl Coenzyme A (HMGCoA) in a series of reactions. The second pathway, known as the mevalonate-independent pathway, produces IPP from 1-deoxyxylulose-5-phosphate (DXP) in a series of reactions. One of those reactions involves the use of DXP synthase
20 (DXS) to catalyze the condensation of pyruvate and glyceraldehyde-3-phosphate to form DXP.

Once made, IPP can be used to make various isoprenoid compounds. Specifically, enzymes known as polyprenyl diphosphate synthases catalyze polymerization reactions that combine IPP and DMAPP to form compounds known as
25 polyprenyl diphosphates. For example, decaprenyl diphosphate synthase (DDS) catalyzes the consecutive condensation of IPP with allylic diphosphates to produce decaprenyl diphosphate. Decaprenyl diphosphate is a polyprenyl diphosphate that can be used to form the side chain of a ubiquinone known as CoQ(10). Other polyprenyl diphosphate synthases include, without limitation, farnesyl-, geranyl-, and octaprenyl diphosphate
30 synthases.

SUMMARY

The invention relates to methods and materials involved in the production of isoprenoid compounds. Specifically, the invention provides nucleic acid molecules, polypeptides, host cells, and methods that can be used to produce isoprenoid compounds.

5 Isoprenoid compounds are both biologically and commercially important. For example, the nutritional industry uses isoprenoid compounds as nutritional supplements, while the perfume industry uses isoprenoid compounds as fragrances. The nucleic acid molecules described herein can be used to engineer host cells having the ability to produce particular isoprenoid compounds. The polypeptides described herein can be used in cell-free

10 systems to make particular isoprenoid compounds. The host cells described herein can be used in culture systems to produce large quantities of particular isoprenoid compounds.

In general, the invention features an isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:1 over the length, wherein the point defined by the length and the percent identity is within

15 the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (3626, 100), point B has coordinates (3626, 65), point C has coordinates (50, 65), and point D has coordinates (12, 100). The point B can have coordinates (3626, 85). The point C can have coordinates (100, 65). The point C can have coordinates (50, 85). The point D can have coordinates (15, 100). The nucleic acid sequence can encode a

20 polypeptide. The polypeptide can have DXS activity. The nucleic acid sequence can be as set forth in SEQ ID NO:1.

In one embodiment, the invention features an isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:2 over the length, wherein the point defined by the length and the percent

25 identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1926, 100), point B has coordinates (1926, 65), point C has coordinates (50, 65), and point D has coordinates (12, 100). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DXS activity.

In another embodiment, the invention features an isolated nucleic acid containing

30 a nucleic acid sequence, wherein the nucleic acid sequence encodes a polypeptide containing an amino acid sequence, wherein the amino acid sequence has a length and a

percent identity to the sequence set forth in SEQ ID NO:3 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (641, 100), point B has coordinates (641, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100). The polypeptide can have DXS activity.

Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:37 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1990, 100), point B has coordinates (1990, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100). The point B can have coordinates (1990, 85). The point C can have coordinates (100, 55). The point C can have coordinates (50, 85). The point D can have coordinates (20, 100). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DDS activity. The nucleic acid sequence can be as set forth in SEQ ID NO:37.

Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:38 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1002, 100), point B has coordinates (1002, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DDS activity.

Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence, wherein the nucleic acid sequence encodes a polypeptide containing an amino acid sequence, wherein the amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:39 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (333, 100), point B has coordinates (333, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100). The polypeptide can have DDS activity.

Another embodiment of the invention features an isolated nucleic acid containing

a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:40 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1833, 100), point B has coordinates (1833, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100). The point B can have coordinates (1833, 85). The point C can have coordinates (100, 65). The point C can have coordinates (50, 85). The point D can have coordinates (20, 100). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DDS activity. The nucleic acid sequence can be as set forth in SEQ ID NO:40.

10 Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:41 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1014, 100), point B has coordinates (1014, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DDS activity.

15 Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence, wherein the nucleic acid sequence encodes a polypeptide containing an amino acid sequence, wherein the amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:42 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (337, 100), point B has coordinates (337, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100). The polypeptide can have DDS activity.

25 Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:95 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (2017, 100), point B has coordinates (2017, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100). The point B can have coordinates (2017, 85). The point C can have coordinates (100, 65). The point C can have coordinates (50, 30

85). The point D can have coordinates (20, 100). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DXR activity. The nucleic acid sequence can be as set forth in SEQ ID NO:95.

Another embodiment of the invention features an isolated nucleic acid containing
5 a nucleic acid sequence having a length and a percent identity to the sequence set forth in
SEQ ID NO:96 over the length, wherein the point defined by the length and the percent
identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A
has coordinates (1161, 100), point B has coordinates (1161, 65), point C has coordinates
(50, 65), and point D has coordinates (16, 100). The nucleic acid sequence can encode a
10 polypeptide. The polypeptide can have DXR activity.

Another embodiment of the invention features an isolated nucleic acid containing
a nucleic acid sequence, wherein the nucleic acid sequence encodes a polypeptide
containing an amino acid sequence, wherein the amino acid sequence has a length and a
percent identity to the sequence set forth in SEQ ID NO:97 over the length, wherein the
15 point defined by the length and the percent identity is within the area defined by points A,
B, C, and D of Figure 26, wherein point A has coordinates (386, 100), point B has
coordinates (386, 65), point C has coordinates (25, 65), and point D has coordinates (5,
100). The polypeptide can have DXR activity.

Another embodiment of the invention features an isolated nucleic acid containing
20 a nucleic acid sequence of at least 12 nucleotides, wherein the isolated nucleic acid
hybridizes under hybridization conditions to the sense or antisense strand of a nucleic
acid molecule, the sequence of the nucleic acid molecule being the sequence set forth in
SEQ ID NO: 1, 2, 37, 38, 40, 41, 95, or 96. The nucleic acid sequence can be at least 50
nucleotides (e.g., at least 100, 200, 300, 400, 500, or more). The nucleic acid sequence
25 can encode a polypeptide. The polypeptide can have DXS, DDS, or DXR activity.

In another aspect, the invention features a substantially pure polypeptide
containing an amino acid sequence, wherein the amino acid sequence has a length and a
percent identity to the sequence set forth in SEQ ID NO:3 over the length, wherein the
point defined by the length and the percent identity is within the area defined by points A,
30 B, C, and D of Figure 26, wherein point A has coordinates (641, 100), point B has
coordinates (641, 65), point C has coordinates (25, 65), and point D has coordinates (5,

100). The polypeptide can have DXS activity.

In another embodiment, the invention features a substantially pure polypeptide containing an amino acid sequence, wherein the amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:39 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (333, 100), point B has coordinates (333, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100). The polypeptide can have DDS activity.

Another embodiment of the invention features a substantially pure polypeptide containing an amino acid sequence, wherein the amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:42 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (337, 100), point B has coordinates (337, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100). The polypeptide can have DDS activity.

Another embodiment of the invention features a substantially pure polypeptide containing an amino acid sequence, wherein the amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:97 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (386, 100), point B has coordinates (386, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100). The polypeptide can have DXR activity.

Another aspect of the invention features a host cell containing an isolated nucleic acid of claim 1, 9, 12, 14, 22, 25, 27, 35, 38, 40, 48, 51, or 53. The host cell can be prokaryotic. The host cell can be a *Rhodobacter*, *Sphingomonas*, or *Escherichia* cell. The host cell can contain an exogenous nucleic acid that encodes a polypeptide having DDS, DXS, ODS, SDS, DXR, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase, 4-diphosphocytidyl-2C-methyl-D-erythritol kinase, or chorismate lyase activity. The host cell can contain an exogenous nucleic acid containing an UbiC sequence or LytB sequence. The host cell can contain an exogenous nucleic acid containing an UbiC sequence and LytB sequence. The host cell can contain a non-functional crtE sequence,

ppsR sequence, or ccoN sequence. The host cell can contain a non-functional crtE sequence, ppsR sequence, and ccoN sequence.

Another embodiment of the invention features a host cell containing an exogenous nucleic acid and a non-functional crtE sequence, ppsR sequence, or ccoN sequence, wherein the exogenous nucleic acid is within a crtE, ppsR, or ccoN locus of the host cell.

Another embodiment of the invention features a host cell containing a genomic deletion, wherein the deletion comprises at least a portion of a crtE sequence, ppsR sequence, or ccoN sequence, and wherein the host cell comprises a non-functional crtE sequence, ppsR sequence, or ccoN sequence.

Another aspect of the invention features a method for increasing production of CoQ(10) in a cell having endogenous DDS activity. The method includes inserting a nucleic acid molecule containing a nucleic acid sequence that encodes a polypeptide having DDS activity into the cell such that production of CoQ(10) is increased. The nucleic acid molecule can contain an isolated nucleic acid of claim 14, 22, 25, 27, 35, 38, or 53. The production of CoQ(10) can be increased at least about 5 percent as compared to a control cell lacking the inserted nucleic acid molecule. The cell can be a *Rhodobacter* or *Sphingomonas* cell. The cell can be a membraneous bacterium or highly membraneous bacterium. The method can also include inserting a second nucleic acid molecule containing a nucleotide sequence that encodes a polypeptide having DXS activity into the cell. The second nucleic acid molecule can contain an isolated nucleic acid of claim 1, 9, or 12.

In another embodiment, the invention features a method for increasing production of CoQ(10) in a cell having endogenous DDS activity. The method includes inserting a nucleic acid molecule containing a nucleic acid sequence that encodes a polypeptide having DXS activity into the cell such that production of CoQ(10) is increased. The production of CoQ(10) can be increased at least about 5 percent as compared to a control cell lacking the inserted nucleic acid molecule. The cell can be a *Rhodobacter* or *Sphingomonas* cell. The nucleic acid molecule can contain an isolated nucleic acid of claim 1, 9, or 12. The cell can be a membraneous bacterium or highly membraneous bacterium. The method can also include inserting a second nucleic acid molecule containing a nucleotide sequence that encodes a polypeptide having DDS activity into the

cell. The second nucleic acid molecule can contain an isolated nucleic acid of claim 14, 22, 25, 27, 35, 38, or 53.

Another embodiment of the invention features a method for increasing production of CoQ(10) in a membraneous bacterium. The method includes inserting a nucleic acid molecule containing a nucleic acid sequence that encodes a polypeptide having DDS
5 activity into the bacterium such that production of CoQ(10) is increased.

Another embodiment of the invention features a method for increasing production of CoQ(10) in a highly membraneous bacterium. The method includes inserting a nucleic acid molecule containing a nucleic acid sequence that encodes a polypeptide having DDS
10 activity into the highly membraneous bacterium such that production of CoQ(10) is increased.

Another embodiment of the invention features a method for making an isoprenoid. The method includes culturing a cell under conditions wherein the cell produces the isoprenoid, wherein the cell contains at least one exogenous nucleic acid that encodes at least one polypeptide, wherein the cell produces more of the isoprenoid than a
15 comparable cell lacking the at least one exogenous nucleic acid. The cell can be a *Rhodobacter* or *Sphingomonas* cell. The isoprenoid can be CoQ(10). The at least one polypeptide can have DDS, DXS, ODS, SDS, DXR, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase, 4-diphosphocytidyl-2C-methyl-D-erythritol kinase, or chorismate
20 lyase activity. The at least one polypeptide can be a UbiC polypeptide or a LytB polypeptide. The cell can contain a non-functional crtE sequence, ppsR sequence, or ccoN sequence. The cell can contain a non-functional crtE sequence, ppsR sequence, and ccoN sequence. The cell can contain a genomic deletion, wherein the deletion contains at least a portion of a crtE sequence, ppsR sequence, or ccoN sequence, and wherein the cell
25 contains a non-functional crtE sequence, ppsR sequence, or ccoN sequence.

Another embodiment of the invention features a method for making an isoprenoid. The method includes culturing a genetically modified cell under conditions wherein the cell produces the isoprenoid. The isoprenoid can be CoQ(10). The cell can contain an exogenous nucleic acid. The cell can contain a genomic deletion.

30 Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this

invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

10

DESCRIPTION OF DRAWINGS

Figure 1 is a diagram of a pathway for producing CoQ(10).

Figure 2 is a listing of a nucleic acid sequence that encodes a *Sphingomonas trueperi* (ATCC 12417) polypeptide having DXS activity (SEQ ID NO:1). The start codon is the ATG at nucleotide number 182, and the stop codon is the TAA at nucleotide number 2107. The probable ribosome binding site is at nucleotide numbers 175-178. This sequence contains an open reading frame as well as 5' and 3' untranslated sequences.

Figure 3 is a listing of a nucleic acid sequence that encodes a *Sphingomonas trueperi* (ATCC 12417) polypeptide having DXS activity (SEQ ID NO:2). This sequence corresponds to the open reading frame.

Figure 4 is a listing of an amino acid sequence of a *Sphingomonas trueperi* (ATCC 12417) polypeptide having DXS activity (SEQ ID NO:3).

Figure 5 is a sequence pile-up of 14 nucleic acid sequences that encode polypeptides having DXS activity. STdxsdna represents the nucleic acid sequence set forth in SEQ ID NO:2; CRdxsdna represents a nucleic acid sequence from *Chlamydomonas reinhardtii* (GenBank accession number AJ007559; SEQ ID NO:4); CJdxsdna represents a nucleic acid sequence from *Campylobacter jejuni* (GenBank accession number AL139074; SEQ ID NO:5); PAdxsdna represents a nucleic acid sequence from *Pseudomonas aeruginosa* (GenBank accession number AE004821; SEQ ID NO:6); LEDxsdna represents a nucleic acid sequence from *Lycopersicon esculentum* (GenBank accession number AF143812; SEQ ID NO:7); MTdxsdna represents a nucleic

acid sequence from *Mycobacterium tuberculosis* (GenBank accession number Z96072; ; SEQ ID NO:8); RSdxs1dna represents a nucleic acid sequence from a *Rhodobacter sphaeroides* dxs1 gene (SEQ ID NO:9); RSdxs2dna represents a nucleic acid sequence from a *Rhodobacter sphaeroides* dxs2 gene (SEQ ID NO:10); SPCCdxsdna represents a nucleic acid sequence from *Synechococcus* PCC6301 (GenBank accession number Y18874; SEQ ID NO:11); ECdxsdna represents a nucleic acid sequence from *Escherichia coli* (GenBank accession number AF035440; SEQ ID NO:12); NMdxsdna represents a nucleic acid sequence from *Neisseria meningitidis* (GenBank accession number AL162753; SEQ ID NO:13); Hldxsdna represents a nucleic acid sequence from *Haemophilus influenza* (GenBank accession number U32822; SEQ ID NO:14); SSdxsdna represents a nucleic acid sequence from *Streptomyces sp.* CL190 (GenBank accession number AB026631; SEQ ID NO:16); and HPdxsdna represents a nucleic acid sequence from *Helicobacter pylori* 26695 (GenBank accession number AE000552; SEQ ID NO:17).

Figure 6 is a sequence pile-up of 21 amino acid sequences of polypeptides having DXS activity. STdxsp represents an amino acid sequence set forth in SEQ ID NO:3; AAdxsp represents an amino acid sequence from *Aquifex aeolicus* (GenBank accession number O67036; SEQ ID NO:18); BSdxsp represents an amino acid sequence from *Bacillus subtilis* (GenBank accession number P54523; SEQ ID NO:19); CRdxsp represents an amino acid sequence from *Chlamydomonas reinhardtii* (GenBank accession number CAA07554; SEQ ID NO:20); CJdxsp represents an amino acid sequence from *Campylobacter jejuni* (GenBank accession number CAB72788; SEQ ID NO:21); PAdxsp represents an amino acid sequence from *Pseudomonas aeruginosa* (GenBank accession number AAG07431; SEQ ID NO:15); LEdxsp represents an amino acid sequence from *Lycopersicon esculentum* (GenBank accession number AAD38941; SEQ ID NO:22); MLdxsp represents an amino acid sequence from *Mycobacterium leprae* (GenBank accession number Q50000; SEQ ID NO:23); MTdxsp represents an amino acid sequence from *Mycobacterium tuberculosis* (GenBank accession number CAB09493; SEQ ID NO:24); RCDxsp represents an amino acid sequence from *Rhodobacter capsulatus* (GenBank accession number P26242; SEQ ID NO:25); RSdxs1p represents an amino acid sequence encoded by a *Rhodobacter sphaeroides* dxs1 gene (SEQ ID NO:26);

RSdxs2p represents an amino acid sequence encoded by a *Rhodobacter sphaeroides* dxs2 gene (SEQ ID NO:27); SPCCdxsp represents an amino acid sequence from *Synechococcus* PCC6301 (GenBank accession number CAB60078; SEQ ID NO:28); SPdxsp represents an amino acid sequence from *Synechocystis* PCC6803 (GenBank
 5 accession number P73067; SEQ ID NO:29); TMdxsp represents an amino acid sequence from *Thermotoga maritima* (GenBank accession number Q9X291; SEQ ID NO:30); ECdxsp represents an amino acid sequence from *Escherichia coli* (GenBank accession number D64771; SEQ ID NO:31); NMdxsp represents an amino acid sequence from *Neisseria meningitidis* (GenBank accession number CAB83880; SEQ ID NO:32); HIdxsp
 10 represents an amino acid sequence from *Haemophilus influenza* (GenBank accession number B64172; SEQ ID NO:33); PFdxsp represents an amino acid sequence from *Plasmodium falciparum* (GenBank accession number AAD03740; SEQ ID NO:34); SSdxsp represents an amino acid sequence from *Streptomyces* sp. CL190 (GenBank accession number BAA85847; SEQ ID NO:35); and HPdxsp represents an amino acid
 15 sequence from *Helicobacter pylori* 26695 (GenBank accession number AAD07422; SEQ ID NO:36).

Figure 7 is a listing of a nucleic acid sequence that encodes a *Rhodobacter sphaeroides* (ATCC 17023) polypeptide having DDS activity (SEQ ID NO:37). The start codon is the ATG at nucleotide number 372, and the stop codon is the TGA at nucleotide
 20 number 1373. The probable ribosome binding site is at nucleotide numbers 363-366. This sequence contains an open reading frame as well as 5' and 3' untranslated sequences.

Figure 8 is a listing of a nucleic acid sequence that encodes a *Rhodobacter sphaeroides* (ATCC 17023) polypeptide having DDS activity (SEQ ID NO:38). This
 25 sequence corresponds to the open reading frame.

Figure 9 is a listing of an amino acid sequence of a *Rhodobacter sphaeroides* (ATCC 17023) polypeptide having DDS activity (SEQ ID NO:39).

Figure 10 is a listing of a nucleic acid sequence that encodes a *Sphingomonas trueperi* (ATCC 12417) polypeptide having DDS activity (SEQ ID NO:40). The start
 30 codon is the ATG at nucleotide number 605, and the stop codon is the TGA at nucleotide number 1618. The probable ribosome binding site is at nucleotide numbers 590-594.

This sequence contains an open reading frame as well as 5' and 3' untranslated sequences.

Figure 11 is a listing of a nucleic acid sequence that encodes a *Sphingomonas trueperi* (ATCC 12417) polypeptide having DDS activity (SEQ ID NO:41). This sequence corresponds to the open reading frame.

Figure 12 is a listing of an amino acid sequence of a *Sphingomonas trueperi* (ATCC 12417) polypeptide having DDS activity (SEQ ID NO:42). This sequence corresponds to the open reading frame.

Figure 13 is a sequence pile-up of five nucleic acid sequences that encode polypeptides having DDS activity. RSddsdna represents the nucleic acid sequence set forth in SEQ ID NO:38; STddsdna represents the nucleic acid sequence set forth in SEQ ID NO:41; SPddsdna represents a nucleic acid sequence from *Schizosaccharomyces pombe* (GenBank accession number D84311; SEQ ID NO:43); GSddsdna represents a nucleic acid sequence from *Gluconobacter suboxydans* (GenBank accession number AB006850; SEQ ID NO:44); and RCddsdna represents a nucleic acid sequence from *Rhodobacter capsulatus* (U.S. Patent No. 6,103,488; SEQ ID NO:45).

Figure 14 is a sequence pile-up of five amino acid sequences of polypeptides having DDS activity. RSddsp represents the amino acid sequence set forth in SEQ ID NO:39; STddsp represents the amino acid sequence set forth in SEQ ID NO:42; GSddsp represents an amino acid sequence from *Gluconobacter suboxydans* (GenBank accession number BAA32241; SEQ ID NO:46); SPddsp represents an amino acid sequence from *Schizosaccharomyces pombe* (GenBank accession number CAB66154; SEQ ID NO:47); and RCddsp represents an amino acid sequence from *Rhodobacter capsulatus* (U.S. Patent No. 6,103,488; SEQ ID NO:48).

Figure 15 is a sequence pile-up of three amino acid sequences of polypeptides having DXS activity. Hpdxsp represents the amino acid sequence set forth in SEQ ID NO:36; Ecdxsp represents the amino acid sequence set forth in SEQ ID NO:31; and Hidxsp represents the amino acid sequence set forth in SEQ ID NO:33.

Figure 16 is a sequence pile-up of four amino acid sequences of polypeptides having DDS, ODS (octaprenyl diphosphate synthase), or SDS (solanesyl diphosphate synthase) activity. Rcsdsp represents an amino acid sequence from *Rhodobacter*

capsulatus having SDS activity (SEQ ID NO:49); Rpodsp represents an amino acid sequence from *Rickettsia prowazeki* having ODS activity (SEQ ID NO:50); Gsddsp represents the amino acid sequence set forth in SEQ ID NO:46; and Ecodsp represents an amino acid sequence from *Escherichia coli ispB* having ODS activity (SEQ ID NO:51).

5 Figure 17 is a sequence pile-up of five amino acid sequences of polypeptides having DDS, ODS, or SDS activity. Rpodsp represents the amino acid sequence set forth in SEQ ID NO:50; Gsddsp represents the amino acid sequence set forth in SEQ ID NO:46; Ecodsp represents the amino acid sequence set forth in SEQ ID NO:51; Hiodsp represents an amino acid sequence from *Haemophilus influenzae* having ODS activity
10 (SEQ ID NO:52); and Rcsdsp represents the amino acid sequence set forth in SEQ ID NO:49.

Figure 18 is a diagram of a construct designated appUC18-SHDXS.

Figure 19 is a diagram of a construct designated appUC18-RSdds.

Figure 20 is a diagram of a construct designated appUC18-SHDDS.

15 Figure 21 is a mass chromatogram obtained from a MG1655 PUC18 specimen.

Figure 22 is a mass chromatogram obtained from a MG1655 PUC18-DDS specimen.

Figure 23 is a mass spectra obtained from a MG1655 PUC18 specimen.

Figure 24 is a mass spectra obtained from a MG1655 PUC18-DDS specimen.

20 Figure 25 is a mass spectra obtained from a MG1655 PUC18-DDS specimen.

Figure 26 is a graph plotting length and percent identity with points A, B, C, and D defining an area indicated by shading.

Figure 27 is a sequence pile-up of seven amino acid sequences of polypeptides having DXR activity. Bsdxrp represents an amino acid sequence from *Bacillus subtilis*
25 (SEQ ID NO:98); Hmdxrp represents an amino acid sequence from *Haemophilus influenzae* (SEQ ID NO:99); Ecdxrp represents an amino acid sequence from *Escherichia coli* (SEQ ID NO:100); Zmdxrp represents an amino acid sequence from *Zymonas mobilis* (SEQ ID NO:101); Sldxrp represents an amino acid sequence from *Synechococcus leopoliensis* (SEQ ID NO:102); Ssdxrp represents an amino acid sequence
30 from *Synechocystis sp. PCC6803* (SEQ ID NO:103); and Mtdxrp represents an amino acid sequence from *Mycobacterium tuberculosis* (SEQ ID NO:104).

Figure 28 is a listing of a nucleic acid sequence that encodes a *Sphingomonas trueperi* polypeptide having DXR activity (SEQ ID NO:95). The start codon is the GTG at either nucleotide number 575 or 578, and the stop codon is the TGA at nucleotide number 1733. This sequence contains an open reading frame as well as 5' and 3'

5 untranslated sequences.

Figure 29 is a listing of a nucleic acid sequence that encodes a *Sphingomonas trueperi* polypeptide having DXR activity (SEQ ID NO:96). This sequence corresponds to the open reading frame.

Figure 30 is a listing of an amino acid sequence of a *Sphingomonas trueperi* polypeptide having DXR activity (SEQ ID NO:97).

Figure 31 is a sequence pile-up of twelve nucleic acid sequences that encode polypeptides having DXR activity. Stdxcrd represents the nucleic acid sequence set forth in SEQ ID NO:96; Padxcd represents a nucleic acid sequence from *Pseudomonas aeruginosa* (SEQ ID NO:105); Zmdxcd represents a nucleic acid sequence from

15 *Zygomonas mobilis* (SEQ ID NO:106); Sgdcxd represents a nucleic acid sequence from *Streptomyces griseolosporeus* (SEQ ID NO:107); Nmdxcd represents a nucleic acid sequence from *Neisseria meningitidis* (SEQ ID NO:108); Ecdxcd represents a nucleic acid sequence from *Escherishia coli* (SEQ ID NO:109); Sldxcd represents a nucleic acid sequence from *Synechococcus leopoliensis* (SEQ ID NO:110); Mldxcd represents a

20 nucleic acid sequence from *Mycobacterium leprae* (SEQ ID NO:111); Pmdxcd represents a nucleic acid sequence from *Pasteurella multocida* (SEQ ID NO:112); Atdcxd represents a nucleic acid sequence from *Arabidopsis thaliana* (SEQ ID NO:113); Cjdcxd represents a nucleic acid sequence from *Campylobacter jejuni* (SEQ ID NO:114); and Pfdxcd represents a nucleic acid sequence from *Plasmodium falciparum* (SEQ ID NO:115).

Figure 32 is a sequence pile-up of sixteen amino acid sequences of polypeptides having DXR activity. Stdxcrp represents the amino acid sequence set forth in SEQ ID NO:97; Zmdxcrp represents an amino acid sequence from *Zymononas mobilis* (SEQ ID NO:116); Padxcrp represents an amino acid sequence from *Pseudomonas aeruginosa* (SEQ ID NO:117); Ecdxcrp represents an amino acid sequence from *Escherishia coli*

30 (SEQ ID NO:118); Nmdxcrp represents an amino acid sequence from *Neisseria meningitidis* (SEQ ID NO:119); Hidxcrp represents an amino acid sequence from

Haemophilus influenzae (SEQ ID NO:120); Ssdxrp represents an amino acid sequence from *Synechocystis* sp. PCC6803 (SEQ ID NO:121); Pmdxrp represents an amino acid sequence from *Pasteurella multocida* (SEQ ID NO:122); Sldxrp represents an amino acid sequence from *Synechococcus leopoliensis* (SEQ ID NO:123); Sgdxrp represents an amino acid sequence from *Streptomyces griseolosporeus* (SEQ ID NO:124); Bsdxrp represents an amino acid sequence from *Bacillus subtilis* (SEQ ID NO:125); Mldxrp represents an amino acid sequence from *Mycobacterium leprae* (SEQ ID NO:126); Mtdxrp represents an amino acid sequence from *Mycobacterium tuberculosis* (SEQ ID NO:127); Atdxrp represents an amino acid sequence from *Arabidopsis thaliana* (SEQ ID NO:128); Cjdxrp represents an amino acid sequence from *Campylobacter jejuni* (SEQ ID NO:130); and Pfdxrp represents an amino acid sequence from *Plasmodium falciparum* (SEQ ID NO:131).

DETAILED DESCRIPTION

The invention provides methods and materials related to the production of isoprenoids. Specifically, the invention provides isolated nucleic acids, substantially pure polypeptides, host cells, and methods and materials for producing various isoprenoid compounds. For the purpose of this invention, an isoprenoid compound is any compound containing a five-carbon isoprenoid unit. Examples of isoprenoid compounds include, without limitation, carotenoids, isoprenes, sterols, terpenes, and ubiquinones. Such isoprenoid compounds can be used in a wide range of applications. For example, isoprenoid compounds produced as described herein can be used in industrial, pharmaceutical, or cosmetic products.

In general terms, carotenoids are lipophilic pigments typically found in photosynthetic plants and bacteria. Examples of carotenoids include, without limitation, carotenes, xanthophylls, hydrocarbon carotenoids, hydroxy carotenoid derivatives, epoxy carotenoid derivatives, furanoxo carotenoid derivatives, and oxy carotenoid derivatives. Isoprenes are oily hydrocarbons that can be obtained by distilling caoutchouc or guttaipercha. Examples of isoprenes include, without limitation, rubber, vitamin A, and vitamin K. Sterols are steroid-based alcohols typically having a hydrocarbon side-chain of eight to ten carbon atoms at the 17-beta position and a hydroxyl group at the 3-beta

position. Examples of sterols include, without limitation, ergosterol, cholesterol, and stigmasterol. Terpenes are lipid species typically found in plants in great abundance. Examples of terpenes include, without limitation, dolichol, squalene, and limonene. Ubiquinones are 2,3-dimethoxy-5-methylbenzoquinone derivatives having a side chain containing at least one isoprenoid unit. Typically, ubiquinone is referred to as Coenzyme Q (CoQ). In addition, the number of isoprenoid units of a side chain of a particular ubiquinone is used to identify that particular ubiquinone. For example, a ubiquinone with six isoprenoid units is referred to as CoQ(6), while a ubiquinone with ten isoprenoid units is referred to as CoQ(10). It is noted that CoQ(10) also is referred to as ubiquinone. Examples of ubiquinones include, without limitation, CoQ(6), CoQ(8), CoQ(10), and CoQ(12).

Isoprenoid compounds can be pyruvate-derived products. The term "pyruvate-derived product" as used herein refers to any compound that is synthesized from pyruvate within no more than 25 enzymatic steps. Thus, an isoprenoid compound is not a pyruvate-derived product if that isoprenoid compound is synthesized from pyruvate in more than 25 enzymatic steps. An enzymatic step is a single chemical reaction catalyzed by a polypeptide having enzymatic activity. The term "polypeptide having enzymatic activity" as used herein refers to any polypeptide that catalyzes a chemical reaction of other substances without itself being destroyed or altered upon completion of the reaction. Typically, a polypeptide having enzymatic activity catalyzes the formation of one or more products from one or more substrates. Such polypeptides can have any type of enzymatic activity including, without limitation, the enzymatic activity associated with an enzyme such as DXS, DDS, ODS, SDS, DXR (1-deoxy-D-xylulose 5-phosphate reductoisomerase), ispD (4-diphosphocytidyl-2C-methyl-D-erythritol synthase), and ispE (4-diphosphocytidyl-2C-methyl-D-erythritol kinase).

A polypeptide having a particular enzymatic activity can be a polypeptide that is either naturally-occurring or non-naturally-occurring. A naturally-occurring polypeptide is any polypeptide having an amino acid sequence as found in nature, including wild-type and polymorphic polypeptides. Such naturally-occurring polypeptides can be obtained from any species including, without limitation, animal (e.g., mammalian), plant, fungal, and bacterial species. A non-naturally-occurring polypeptide is any polypeptide having

an amino acid sequence that is not found in nature. Thus, a non-naturally-occurring polypeptide can be a mutated version of a naturally-occurring polypeptide, or an engineered polypeptide. For example, a non-naturally-occurring polypeptide having DDS activity can be a mutated version of a naturally-occurring polypeptide having DDS activity that retains at least some DDS activity. A polypeptide can be mutated by, for example, sequence additions, deletions, substitutions, or combinations thereof.

Examples of isoprenoid compounds that are pyruvate-derived products include, without limitation, CoQ(6), CoQ(7), CoQ(8), CoQ(9), CoQ(10), astaxanthin, canthaxanthin, lutein, zeaxanthin, beta-carotene, lycopene, capsanthin, bixin, norbixin, crocetin, zeta-carotene, vitamin E, giberellins, abscisic acid, ergosterol, geraniol, and latex.

As depicted in Figure 1, multiple polypeptide can be used to convert glucose CoQ(10). For example, polypeptides having DXS, DXR, LytB, and DDS activity can be used to convert glucose CoQ(10). Such polypeptides can be obtained and used to make CoQ(10) as described herein.

1. Nucleic acids

The term "nucleic acid" as used herein encompasses both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. The nucleic acid can be double-stranded or single-stranded. Where single-stranded, the nucleic acid can be the sense strand or the antisense strand. In addition, nucleic acid can be circular or linear.

The term "isolated" as used herein with reference to nucleic acid refers to a naturally-occurring nucleic acid that is not immediately contiguous with both of the sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally-occurring genome of the organism from which it is derived. For example, an isolated nucleic acid can be, without limitation, a recombinant DNA molecule of any length, provided one of the nucleic acid sequences normally found immediately flanking that recombinant DNA molecule in a naturally-occurring genome is removed or absent. Thus, an isolated nucleic acid includes, without limitation, a recombinant DNA that exists as a separate molecule (e.g., a cDNA or a genomic DNA

fragment produced by PCR or restriction endonuclease treatment) independent of other sequences as well as recombinant DNA that is incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or into the genomic DNA of a prokaryote or eukaryote. In addition, an isolated nucleic acid can include a recombinant DNA molecule that is part of a hybrid or fusion nucleic acid sequence.

The term "isolated" as used herein with reference to nucleic acid also includes any non-naturally-occurring nucleic acid since non-naturally-occurring nucleic acid sequences are not found in nature and do not have immediately contiguous sequences in a naturally-occurring genome. For example, non-naturally-occurring nucleic acid such as an engineered nucleic acid is considered to be isolated nucleic acid. Engineered nucleic acid can be made using common molecular cloning or chemical nucleic acid synthesis techniques. Isolated non-naturally-occurring nucleic acid can be independent of other sequences, or incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or the genomic DNA of a prokaryote or eukaryote. In addition, a non-naturally-occurring nucleic acid can include a nucleic acid molecule that is part of a hybrid or fusion nucleic acid sequence.

It will be apparent to those of skill in the art that a nucleic acid existing among hundreds to millions of other nucleic acid molecules within, for example, cDNA or genomic libraries, or gel slices containing a genomic DNA restriction digest is not to be considered an isolated nucleic acid.

The term "exogenous" as used herein with reference to nucleic acid and a particular cell refers to any nucleic acid that does not originate from that particular cell as found in nature. Thus, all non-naturally-occurring nucleic acid is considered to be exogenous to a cell once introduced into the cell. It is important to note that non-naturally-occurring nucleic acid can contain nucleic acid sequences or fragments of nucleic acid sequences that are found in nature provided the nucleic acid as a whole does not exist in nature. For example, a nucleic acid molecule containing a genomic DNA sequence within an expression vector is non-naturally-occurring nucleic acid, and thus is exogenous to a cell once introduced into the cell, since that nucleic acid molecule as a whole (genomic DNA plus vector DNA) does not exist in nature. Thus, any vector,

autonomously replicating plasmid, or virus (e.g., retrovirus, adenovirus, or herpes virus) that as a whole does not exist in nature is considered to be non-naturally-occurring nucleic acid. It follows that genomic DNA fragments produced by PCR or restriction endonuclease treatment as well as cDNAs are considered to be non-naturally-occurring nucleic acid since they exist as separate molecules not found in nature. It also follows that any nucleic acid containing a promoter sequence and polypeptide-encoding sequence (e.g., cDNA or genomic DNA) in an arrangement not found in nature is non-naturally-occurring nucleic acid.

Nucleic acid that is naturally-occurring can be exogenous to a particular cell. For example, an entire chromosome isolated from a cell of person X is an exogenous nucleic acid with respect to a cell of person Y once that chromosome is introduced into Y's cell.

The invention provides isolated nucleic acid that contains a nucleic acid sequence having (1) a length, and (2) a percent identity to an identified nucleic acid sequence over that length. The invention also provides isolated nucleic acid that contains a nucleic acid sequence encoding a polypeptide that contains an amino acid sequence having (1) a length, and (2) a percent identity to an identified amino acid sequence over that length. Typically, the identified nucleic acid or amino acid sequence is a sequence referenced by a particular sequence identification number, and the nucleic acid or amino acid sequence being compared to the identified sequence is referred to as the target sequence. For example, an identified sequence can be the sequence set forth in SEQ ID NO: 1.

A length and percent identity over that length for any nucleic acid or amino acid sequence is determined as follows. First, a nucleic acid or amino acid sequence is compared to the identified nucleic acid or amino acid sequence using the BLAST 2 Sequences (Bl2seq) program from the stand-alone version of BLASTZ containing BLASTN version 2.0.14 and BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from the University of Wisconsin library as well as at www.fr.com or www.ncbi.nlm.nih.gov. Instructions explaining how to use the Bl2seq program can be found in the readme file accompanying BLASTZ. Bl2seq performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. To compare two nucleic acid sequences, the options are set as

follows: -i is set to a file containing the first nucleic acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second nucleic acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastn; -o is set to any desired file name (e.g., C:\output.txt); -q is set to -1; -r is set to 2; and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two sequences: C:\Bl2seq -i c:\seq1.txt -j c:\seq2.txt -p blastn -o c:\output.txt -q -1 -r 2. To compare two amino acid sequences, the options of Bl2seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\Bl2seq -i c:\seq1.txt -j c:\seq2.txt -p blastp -o c:\output.txt. If the target sequence shares homology with any portion of the identified sequence, then the designated output file will present those regions of homology as aligned sequences. If the target sequence does not share homology with any portion of the identified sequence, then the designated output file will not present aligned sequences. Once aligned, a length is determined by counting the number of consecutive nucleotides or amino acid residues from the target sequence presented in alignment with sequence from the identified sequence starting with any matched position and ending with any other matched position. A matched position is any position where an identical nucleotide or amino acid residue is presented in both the target and identified sequence. Gaps presented in the target sequence are not counted since gaps are not nucleotides or amino acid residues. Likewise, gaps presented in the identified sequence are not counted since target sequence nucleotides or amino acid residues are counted, not nucleotides or amino acid residues from the identified sequence.

The percent identity over a determined length is determined by counting the number of matched positions over that length and dividing that number by the length followed by multiplying the resulting value by 100. For example, if (1) a 1000 nucleotide target sequence is compared to the sequence set forth in SEQ ID NO:1, (2) the Bl2seq program presents 200 nucleotides from the target sequence aligned with a region of the

sequence set forth in SEQ ID NO: 1 where the first and last nucleotides of that 200 nucleotide region are matches, and (3) the number of matches over those 200 aligned nucleotides is 180, then the 1000 nucleotide target sequence contains a length of 200 and a percent identity over that length of 90 (i.e. $180 \div 200 * 100 = 90$).

5 It will be appreciated that a single nucleic acid or amino acid target sequence that aligns with an identified sequence can have many different lengths with each length having its own percent identity. For example, a target sequence containing a 20 nucleotide region that aligns with an identified sequence as follows has many different lengths including those listed in Table 1.

10	1	20
Target Sequence:	AGGTCGTGTACTGTCAGTCA	
Identified Sequence:	ACGTGGTGAAGTCCAGTGA	

15 Table I.

Starting Position	Ending Position	Length	Matched Positions	Percent Identity
1	20	20	15	75.0
1	18	18	14	77.8
1	15	15	11	73.3
6	20	15	12	80.0
6	17	12	10	83.3
6	15	10	8	80.0
8	20	13	10	76.9
8	16	9	7	77.8

It is noted that the percent identity value is rounded to the nearest tenth. For example, 78.11, 78.12, 78.13, and 78.14 is rounded down to 78.1, while 78.15, 78.16, 78.17, 78.18, and 78.19 is rounded up to 78.2. It is also noted that the length value will always be an integer.

The invention provides an isolated nucleic acid containing a nucleic acid sequence that has at least one length and percent identity over that length as determined above such that the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26. In addition, the invention provides an isolated nucleic

acid containing a nucleic acid sequence that encodes a polypeptide containing an amino acid sequence that has at least one length and percent identity over that length as determined above such that the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26. The point defined by a length and percent identity over that length is that point on the X/Y coordinate of Figure 26 where the X axis is the length and the Y axis is the percent identity. Thus, the point defined by a nucleic acid sequence with a length of 200 and a percent identity of 90 has coordinates (200, 90). For the purpose of this invention, any point that falls on point A, B, C, or D is considered within the area defined by points A, B, C, and D of Figure 26. Likewise, any point that falls on a line that defines the area defined by points A, B, C, and D is considered within the area defined by points A, B, C, and D of Figure 26.

It will be appreciated that the term "the area defined by points A, B, C, and D of Figure 26" as used herein refers to that area defined by the lines that connect point A with point B, point B with point C, point C with point D, and point D with point A. Points A, B, C, and D can define an area having any shape defined by four points (e.g., square, rectangle, or rhombus). In addition, two or more points can have the same coordinates. For example, points B and C can have identical coordinates. In this case, the area defined by points A, B, C, and D of Figure 26 is triangular. If three points have identical coordinates, then the area defined by points A, B, C, and D of Figure 26 is a line. In this case, any point that falls on that line would be considered within the area defined by points A, B, C, and D of Figure 26. If all four points have identical coordinates, then the area defined by points A, B, C, and D of Figure 26 is a point. In all cases, simple algebraic equations can be used to determine whether a point is within the area defined by points A, B, C, and D of Figure 26.

It is noted that Figure 26 is a graphical representation presenting possible positions of points A, B, C, and D. The shaded area illustrated in Figure 26 represents one possible example, while the arrows indicate that other positions for points A, B, C, and D are possible. In fact, points A, B, C, and D can have any X coordinate and any Y coordinate. For example, point A can have an X coordinate equal to the number of nucleotides or amino acid residues in an identified sequence, and a Y coordinate of 100. Point B can have an X coordinate equal to the number of nucleotides or amino acid

residues in an identified sequence, and a Y coordinate less than or equal to 100 (e.g., 50, 55, 65, 70, 75, 80, 85, 90, 95, and 99). Point C can have an X coordinate equal to a percent (e.g., 1, 2, 5, 10, 15, or more percent) of the number of nucleotides or amino acid residues in an identified sequence, and a Y coordinate less than or equal to 100 (e.g., 50, 55, 65, 70, 75, 80, 85, 90, 95, and 99). Point D can have an X coordinate equal to the length of a typical PCR primer (e.g., 12, 13, 14, 15, 16, 17, or more) or antigenic polypeptide (e.g., 5, 6, 7, 8, 9, 10, 11, 12, or more), and a Y coordinate less than or equal to 100 (e.g., 50, 55, 65, 70, 75, 80, 85, 90, 95, and 99).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:1 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 3626, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 3626, and a Y coordinate greater than or equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 3626, 3600, 3500, 3000, 2500, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 3626, 3600, 3500, 3000, 2500, or less; and the Y coordinate for point B can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (3626, 100), point B can be (3626, 95), point C can be (1900, 95), and point D can be (1900, 100).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:2 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 1926, and a Y coordinate less than or equal to 100; where

point B has an X coordinate less than or equal to 1926, and a Y coordinate greater than or equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 1926, 1900, 1850, 1800, 1750, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 1926, 1900, 1850, 1800, 1750, or less; and the Y coordinate for point B can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (1926, 100), point B can be (1926, 95), point C can be (1000, 95), and point D can be (1000, 100).

An isolated nucleic acid containing a nucleic acid sequence that encodes a polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:3 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 641, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 641, and a Y coordinate greater than or equal to 50; where point C has an X coordinate greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 641, 635, 630, 625, 620, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 641, 635, 630, 625, 620, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (641, 100), point B can be (641, 95), point C can be

(400, 95), and point D can be (400, 100).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:37 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 1990, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 1990, and a Y coordinate greater than or equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 1990, 1950, 1900, 1850, 1800, 1750, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 1990, 1950, 1900, 1850, 1800, 1750, or less; and the Y coordinate for point B can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (1990, 100), point B can be (1990, 95), point C can be (1000, 95), and point D can be (1000, 100).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:38 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 1002, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 1002, and a Y coordinate greater than or equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 1002, 950, 900, 850, 800, 750, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 1002, 950, 900, 850, 800, 750, or less; and the Y coordinate for point B can be 65, 70,

75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (1002, 100), point B can be (1002, 95), point C can be (500, 95), and point D can be (500, 100).

An isolated nucleic acid containing a nucleic acid sequence that encodes a polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:39 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 333, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 333, and a Y coordinate greater than or equal to 50; where point C has an X coordinate greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 333, 330, 325, 320, 315, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 333, 330, 325, 320, 315, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (333, 100), point B can be (333, 95), point C can be (150, 95), and point D can be (150, 100).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:40 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 1833, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 1833, and a Y coordinate greater than or

equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 1833, 1800, 1750, 1700, 1650, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 1833, 1800, 1750, 1700, 1650, or less; and the Y coordinate for point B can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (1833, 100), point B can be (1833, 95), point C can be (900, 95), and point D can be (900, 100).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:41 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 1014, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 1014, and a Y coordinate greater than or equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 1014, 950, 900, 800, 700, 600, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 1014, 950, 900, 800, 700, 600, or less; and the Y coordinate for point B can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (1014, 100), point B can be (1014, 95), point C can be (500, 95), and point D can be (500, 100).

An isolated nucleic acid containing a nucleic acid sequence that encodes a

polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:42 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less
5 than or equal to 337, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 337, and a Y coordinate greater than or equal to 50; where point C has an X coordinate greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be
10 337, 335, 330, 325, 320, 315, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 337, 335, 330, 325, 320, 315, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90,
15 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (337, 100), point B can be (337, 95), point C can be (150, 95), and point D can be (150, 100).

An isolated nucleic acid containing a nucleic acid sequence having a length and a
20 percent identity to the sequence set forth in SEQ ID NO:95 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 2017, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 2017, and a Y coordinate greater than or
25 equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 2017, 2000, 1900, 1950, 1800, 1700, 1600, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for
30 point B can be 2017, 2000, 1900, 1950, 1800, 1700, 1600, or less; and the Y coordinate for point B can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C

can be 50, 60, 70, 80, 90, 100, 150, 200, 500, 1000, 1500, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 250, 500, 1000, 1500, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one
5 embodiment, point A can be (2017, 100), point B can be (2017, 95), point C can be (1800, 95), and point D can be (1800, 100).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:96 over that length is within the scope of the invention provided the point defined by that length and percent identity is
10 within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 1161, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 1161, and a Y coordinate greater than or equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater
15 than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 1161, 1050, 1000, 950, 900, 800, 700, 600, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 1161, 1050, 1000, 950, 900, 800, 700, 600, or less; and the Y coordinate for point B can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C
20 can be 50, 60, 70, 80, 90, 100, 150, 200, 250, 500, 1000, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 250, 500, 1000, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (1161, 100), point B can be (1161, 95), point C can be
25 (1000, 95), and point D can be (1000, 100).

An isolated nucleic acid containing a nucleic acid sequence that encodes a polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:97 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area
30 defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 386, and a Y coordinate less than or equal to 100; where point B has an X

coordinate less than or equal to 386, and a Y coordinate greater than or equal to 50; where point C has an X coordinate greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be
5 386, 380, 375, 370, 375, 360, 365, 350, 325, 300, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 386, 380, 375, 370, 375, 360, 365, 350, 325, 300, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be
10 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 350, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, 200, 300, 350, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (386, 100), point B can be (386, 95), point C can be (350, 95), and point D can be (350, 100).

15 The invention also provides isolated nucleic acid that is at least about 12 bases in length (e.g., at least about 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 100, 250, 500, 750, 1000, 1500, 2000, 3000, 4000, or 5000 bases in length) and hybridizes, under hybridization conditions, to the sense or antisense strand of a nucleic acid having the sequence set forth in SEQ ID NO:1, 2, 37, 38, 40, 41, 95, or 96. The hybridization
20 conditions can be moderately or highly stringent hybridization conditions.

For the purpose of this invention, moderately stringent hybridization conditions mean the hybridization is performed at about 42°C in a hybridization solution containing 25 mM KPO₄ (pH 7.4), 5X SSC, 5X Denhart's solution, 50 µg/mL denatured, sonicated salmon sperm DNA, 50% formamide, 10% Dextran sulfate, and 1-15 ng/mL probe (about
25 5×10^7 cpm/µg), while the washes are performed at about 50°C with a wash solution containing 2X SSC and 0.1% sodium dodecyl sulfate.

Highly stringent hybridization conditions mean the hybridization is performed at about 42°C in a hybridization solution containing 25 mM KPO₄ (pH 7.4), 5X SSC, 5X Denhart's solution, 50 µg/mL denatured, sonicated salmon sperm DNA, 50% formamide,
30 10% Dextran sulfate, and 1-15 ng/mL probe (about 5×10^7 cpm/µg), while the washes are performed at about 65°C with a wash solution containing 0.2X SSC and 0.1% sodium

dodecyl sulfate.

Isolated nucleic acid within the scope of the invention can be obtained using any method including, without limitation, common molecular cloning and chemical nucleic acid synthesis techniques. For example, PCR can be used to obtain an isolated nucleic acid containing a nucleic acid sequence sharing similarity to the sequence set forth in SEQ ID NO:1, 2, 37, 38, 40, 41, 95, or 96. PCR refers to a procedure or technique in which target nucleic acid is amplified in a manner similar to that described in U.S. Patent No. 4,683,195, and subsequent modifications of the procedure described therein. Generally, sequence information from the ends of the region of interest or beyond are used to design oligonucleotide primers that are identical or similar in sequence to opposite strands of a potential template to be amplified. Using PCR, a nucleic acid sequence can be amplified from RNA or DNA. For example, a nucleic acid sequence can be isolated by PCR amplification from total cellular RNA, total genomic DNA, and cDNA as well as from bacteriophage sequences, plasmid sequences, viral sequences, and the like. When using RNA as a source of template, reverse transcriptase can be used to synthesize complementary DNA strands.

An isolated nucleic acid within the scope of the invention also can be obtained by mutagenesis. For example, an isolated nucleic acid containing a sequence set forth in SEQ ID NO:1, 2, 37, 38, 40, 41, 95, or 96 can be mutated using common molecular cloning techniques (e.g., site-directed mutagenesis). Possible mutations include, without limitation, deletions, insertions, and substitutions, as well as combinations of deletions, insertions, and substitutions.

In addition, nucleic acid and amino acid databases (e.g., GenBank[®]) can be used to obtain an isolated nucleic acid within the scope of the invention. For example, any nucleic acid sequence having some homology to a sequence set forth in SEQ ID NO:1, 2, 37, 38, 40, 41, 95, or 96, or any amino acid sequence having some homology to a sequence set forth in SEQ ID NO:3, 39, 42, or 97 can be used as a query to search GenBank[®].

Further, nucleic acid hybridization techniques can be used to obtain an isolated nucleic acid within the scope of the invention. Briefly, any nucleic acid having some homology to a sequence set forth in SEQ ID NO:1, 2, 37, 38, 40, 41, 95, or 96 can be

used as a probe to identify a similar nucleic acid by hybridization under conditions of moderate to high stringency. Once identified, the nucleic acid then can be purified, sequenced, and analyzed to determine whether it is within the scope of the invention as described herein.

5 Hybridization can be done by Southern or Northern analysis to identify a DNA or RNA sequence, respectively, that hybridizes to a probe. The probe can be labeled with a biotin, digoxigenin, an enzyme, or a radioisotope such as ^{32}P . The DNA or RNA to be analyzed can be electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the
10 probe using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook *et al.*, (1989) Molecular Cloning, second edition, Cold Spring harbor Laboratory, Plainview, NY. Typically, a probe is at least about 20 nucleotides in length. For example, a probe corresponding to a 20 nucleotide sequence set forth in SEQ ID NO:1, 2, 37, 38, 40, 41, 95, or 96 can be used to identify an identical or similar nucleic
15 acid. In addition, probes longer or shorter than 20 nucleotides can be used.

 The invention provides isolated nucleic acid that contains the entire nucleic acid sequence depicted in Figure 2, 3, 7, 8, 10, 11, 28, or 29. In addition, the invention provides isolated nucleic acid that contains a portion of the nucleic acid sequence depicted in Figure 2, 3, 7, 8, 10, 11, 28, or 29. For example, the invention provides
20 isolated nucleic acid that contains a 15 nucleotide sequence identical to any 15 nucleotide sequence depicted in Figure 2, 3, 7, 8, 10, 11, 28, or 29 including, without limitation, the sequence starting at nucleotide number 1 and ending at nucleotide number 15, the sequence starting at nucleotide number 2 and ending at nucleotide number 16, the sequence starting at nucleotide number 3 and ending at nucleotide number 17, and so
25 forth. It will be appreciated that the invention also provides isolated nucleic acid that contains a nucleotide sequence that is greater than 15 nucleotides (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more nucleotides) in length and identical to any portion of the sequence depicted in Figure 2, 3, 7, 8, 10, 11, 28, or 29. For example, the invention provides isolated nucleic acid that contains a 25 nucleotide sequence identical
30 to any 25 nucleotide sequence depicted in Figure 2, 3, 7, 8, 10, 11, 28, or 29 including, without limitation, the sequence starting at nucleotide number 1 and ending at nucleotide

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variations. For example, the STdxsdna sequence can contain one variation provided in Figure 5 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 5. It is noted that the full-length nucleic acid sequences depicted in Figure 5 can encode polypeptides having DXS activity. It also is noted that
5 the nucleic acid sequence depicted in Figure 2 contains the nucleic acid sequence depicted in Figure 3.

Figure 13 depicts the nucleic acid sequence depicted in Figure 8 (designated RSddsdna) and the nucleic acid sequence depicted in Figure 11 (designated STddsdna) aligned with each other as well as aligned with three other nucleic acid sequences.

10 Examples of variations of the RSddsdna sequence include, without limitation, any variation of the RSddsdna sequence provided in Figure 13. Examples of variations of the STddsdna sequence include, without limitation, any variation of the STddsdna sequence provided in Figure 13. Such variations are provided in Figure 13 in that a comparison of the nucleotide (or lack thereof) at a particular position of the RSddsdna sequence or the
15 STddsdna sequence with the nucleotide (or lack thereof) at the same position of any of the other nucleic acid sequences depicted in Figure 13 provides a list of specific changes for the RSddsdna sequence and the STddsdna sequence. For example, the "a" at position 511 of the RSddsdna sequence or the "a" at position 756 of the STddsdna sequence can be substituted with an "t" as indicated in Figure 13. Again, it will be appreciated that the
20 RSddsdna sequence as well as the STddsdna sequence can contain any number of variations as well as any combination of types of variations. For example, the RSddsdna sequence can contain one variation provided in Figure 13 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 13. Likewise, the STddsdna sequence can contain one variation provided in Figure 13 or
25 more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 13. It is noted that the full-length nucleic acid sequences depicted in Figure 13 can encode polypeptides having DDS activity. It also is noted that the nucleic acid sequence depicted in Figure 7 contains the nucleic acid sequence depicted in Figure 8 and that the nucleic acid sequence depicted in Figure 10 contains the nucleic acid
30 sequence depicted in Figure 11.

The nucleic acid sequence depicted in Figure 7 contains a nucleic acid sequence

that encodes a *R. sphaeroides* (ATCC 17023) polypeptide having DDS activity. Another variant of this nucleic acid sequence is the nucleic acid sequence of a clone isolated from *R. sphaeroides* (ATCC 35053). Briefly, a *R. sphaeroides* (ATCC 35053) clone was identified and found to contain a sequence identical to the nucleic acid sequence depicted in Figure 7 with the following three exceptions. The *R. sphaeroides* (ATCC 35053) clone has a "t" at position 885 rather than a "c", a "c" inserted after the "c" at position 1620, and a "c" inserted after the "c" at position 1733.

The nucleic acid depicted in Figure 8 also contains a nucleic acid sequence that encodes a *R. sphaeroides* (ATCC 17023) polypeptide having DDS activity. Another variant of this nucleic acid sequence is the nucleic acid sequence of a clone isolated from *R. sphaeroides* (ATCC 35053). Briefly, a *R. sphaeroides* (ATCC 35053) clone was identified and found to contain a sequence identical to the nucleic acid sequence depicted in Figure 8 with the following exception. The *R. sphaeroides* (ATCC 35053) clone has a "t" at position 514 rather than a "c".

Figure 31 depicts the nucleic acid sequence depicted in Figure 29 (designated Stdxcrcds) aligned with eleven other nucleic acid sequences. Examples of variations of the Stdxcrcds sequence include, without limitation, any variation of the Stdxcrcds sequence provided in Figure 31. Such variations are provided in Figure 31 in that a comparison of the nucleotide (or lack thereof) at a particular position of the Stdxcrcds sequence with the nucleotide (or lack thereof) at the same position of any of the other nucleic acid sequences depicted in Figure 31 provides a list of specific changes for the Stdxcrcds sequence. Again, it will be appreciated that the Stdxcrcds sequence can contain any number of variations as well as any combination of types of variations. For example, the Stdxcrcds sequence can contain one variation provided in Figure 31 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 31. It is noted that the full-length nucleic acid sequences depicted in Figure 31 can encode polypeptides having DXR activity. It also is noted that the nucleic acid sequence depicted in Figure 29 contains the nucleic acid sequence depicted in Figure 28.

The invention also provides isolated nucleic acid that contains a variant of a portion of the nucleic acid sequence depicted in Figure 2, 3, 7, 8, 10, 11, 28, or 29 as described herein.

The invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes the entire amino acid sequence depicted in Figure 4, 9, 12, or 30. In addition, the invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes a portion of the amino acid sequence depicted in Figure 4, 9, 12, or 30. For example, the invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes a 15 amino acid sequence identical to any 15 amino acid sequence depicted in Figure 4, 9, 12, or 30 including, without limitation, the sequence starting at amino acid residue number 1 and ending at amino acid residue number 15, the sequence starting at amino acid residue number 2 and ending at amino acid residue number 16, the sequence starting at amino acid residue number 3 and ending at amino acid residue number 17, and so forth. It will be appreciated that the invention also provides isolated nucleic acid that contains a nucleic acid sequence that encodes an amino acid sequence that is greater than 15 amino acid residues (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more amino acid residues) in length and identical to any portion of the sequence depicted in Figure 4, 9, 12, or 30. For example, the invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes a 25 amino acid sequence identical to any 25 amino acid sequence depicted in Figure 4, 9, 12, or 30 including, without limitation, the sequence starting at amino acid residue number 1 and ending at amino acid residue number 25, the sequence starting at amino acid residue number 2 and ending at amino acid residue number 26, the sequence starting at amino acid residue number 3 and ending at amino acid residue number 27, and so forth. Additional examples include, without limitation, isolated nucleic acids that contain a nucleic acid sequence that encodes an amino acid sequence that is 50 or more amino acid residues (e.g., 100, 150, 200, 250, 300, 350, or more amino acid residues) in length and identical to any portion of the sequence depicted in Figure 4, 9, 12, or 30. Such isolated nucleic acids can include, without limitation, those isolated nucleic acids containing a nucleic acid sequence that encodes an amino acid sequence represented in a single line of sequence depicted in Figure 4, 9, 12, or 30 since each line of sequence depicted in these figures, with the exception of the last line, provides a 50 amino acid sequence.

In addition, the invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes an amino acid sequence having a variation of the amino acid

sequence depicted in Figure 4, 9, 12, or 30. For example, the invention provides isolated nucleic acid containing a nucleic acid sequence encoding an amino acid sequence depicted in Figure 4, 9, 12, or 30 that contains a single insertion, a single deletion, a single substitution, multiple insertions, multiple deletions, multiple substitutions, or any combination thereof (e.g., single deletion together with multiple insertions). The invention provides multiple examples of isolated nucleic acid containing a nucleic acid sequence encoding an amino acid sequence having a variation of an amino acid sequence depicted in Figure 4, 9, 12, or 30.

Figure 6 depicts the amino acid sequence depicted in Figure 4 (designated STdxsp) aligned with 20 other amino acid sequences. Examples of variations of the STdxsp sequence include, without limitation, any variation of the STdxsp sequence provided in Figure 6. Such variations are provided in Figure 6 in that a comparison of the amino acid residue (or lack thereof) at a particular position of the STdxsp sequence with the amino acid residue (or lack thereof) at the same position of any of the other 20 amino acid sequences depicted in Figure 6 provides a list of specific changes for the STdxsp sequence. For example, the "t" at position 1148 of the STdxsp sequence can be substituted with an "s" as indicated in Figure 6. As also indicated in Figure 6, the "f" at position 575 of the STdxsp sequence can be substituted with an "m", "a", "l", "i", "y", or "v". For Figure 6, the nucleic acid numbering of Figure 2 is used to number the amino acid residue positions of the STdxsp sequence. Thus, the first amino acid residue of the STdxsp sequence starts with number 182 and proceeds in increments of three. It will be appreciated that the STdxsp sequence can contain any number of variations as well as any combination of types of variations. For example, the STdxsp sequence can contain one variation provided in Figure 6 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 6. It is noted that the 21 full-length amino acid sequences depicted in Figure 6 can be polypeptides having DXS activity.

Figure 14 depicts the amino acid sequence depicted in Figure 9 (designated RSddsp) and the amino acid sequence depicted in Figure 12 (designated STddsp) aligned with each other as well as aligned with three other amino acid sequences. For Figure 14, the nucleic acid numbering of Figure 7 is used to number the amino acid residue positions of the RSddsp sequence, and the nucleic acid numbering of Figure 10 is used to number

the amino acid residue positions of the STddsp sequence. Thus, the first amino acid residue of the RSddsp and STddsp sequences each start with a number other than 1 and proceed in increments of three. Examples of variations of the RSddsp sequence include, without limitation, any variation of the RSddsp sequence provided in Figure 14.

- 5 Examples of variations of the STddsp sequence include, without limitation, any variation of the STddsp sequence provided in Figure 14. Such variations are provided in Figure 14 in that a comparison of the amino acid residue (or lack thereof) at a particular position of the RSddsp sequence or the STddsp sequence with the amino acid residue (or lack thereof) at the same position of any of the other amino acid sequences depicted in Figure 14 provides a list of specific changes for the RSddsp sequence and the STddsp sequence. For example, the "I" at position 762 of the RSddsp sequence or the "I" at position 1007 of the STddsp sequence can be substituted with an "a" as indicated in Figure 14. Again, it will be appreciated that the RSddsp sequence as well as the STddsp sequence can contain any number of variations as well as any combination of types of variations. For example,
- 15 the RSddsp sequence can contain one variation provided in Figure 14 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 14. Likewise, the STddsp sequence can contain one variation provided in Figure 14 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 14. It is noted that the five full-length amino acid sequences depicted in Figure 14 can be polypeptides having DDS activity.
- 20

The amino acid sequence depicted in Figure 9 represents a *R. sphaeroides* (ATCC 17023) polypeptide having DDS activity. Another variant of this amino acid sequence is the amino acid sequence encoded by a clone isolated from *R. sphaeroides* (ATCC 35053). Briefly, a *R. sphaeroides* (ATCC 35053) clone was identified and found to encode an amino acid sequence identical to the amino acid sequence depicted in Figure 9 with the following exception. The *R. sphaeroides* (ATCC 35053) clone has a "y" at position 172 rather than an "h".

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- Figure 32 depicts the amino acid sequence depicted in Figure 30 (designated Stdxxrp) aligned with 15 other amino acid sequences. Examples of variations of the Stdxxrp sequence include, without limitation, any variation of the Stdxxrp sequence provided in Figure 32. Such variations are provided in Figure 32 in that a comparison of
- 30

the amino acid residue (or lack thereof) at a particular position of the Stdxxrp sequence with the amino acid residue (or lack thereof) at the same position of any of the other 15 amino acid sequences depicted in Figure 32 provides a list of specific changes for the Stdxxrp sequence. It will be appreciated that the Stdxxrp sequence can contain any number of variations as well as any combination of types of variations. For example, the Stdxxrp sequence can contain one variation provided in Figure 32 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 32. It is noted that the full-length amino acid sequences depicted in Figure 32 can be polypeptides having DXR activity.

10 The invention also provides isolated nucleic acid containing a nucleic acid sequence encoding an amino acid sequence that contains a variant of a portion of the amino acid sequence depicted in Figure 4, 9, 12, or 30 as described herein.

2. Polypeptides

15 The invention provides substantially pure polypeptides. The term "substantially pure" as used herein with reference to a polypeptide means the polypeptide is substantially free of other polypeptides, lipids, carbohydrates, and nucleic acid with which it is naturally associated. Thus, a substantially pure polypeptide is any polypeptide that is removed from its natural environment and is at least 60 percent pure. A substantially pure polypeptide can be at least about 65, 70, 75, 80, 85, 90, 95, or 99 percent pure. Typically, a substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel.

Any substantially pure polypeptide having an amino acid sequence encoded by a nucleic acid within the scope of the invention is itself within the scope of the invention.

25 In addition, any substantially pure polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:3 over that length as determined herein is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 641, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 641, and a Y coordinate greater than or equal to 50; where point C has an X coordinate

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greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 641, 635, 630, 625, 620, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 641, 635, 630, 625, 620, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (641, 100), point B can be (641, 95), point C can be (400, 95), and point D can be (400, 100).

Any substantially pure polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:39 over that length as determined herein is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 333, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 333, and a Y coordinate greater than or equal to 50; where point C has an X coordinate greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 333, 330, 325, 320, 315, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 333, 330, 325, 320, 315, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (333, 100), point B can be (333, 95), point C can be (150, 95), and point D can be (150, 100).

Any substantially pure polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:42 over that length as determined herein is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 337, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 337, and a Y coordinate greater than or equal to 50; where point C has an X coordinate greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 337, 335, 330, 325, 320, 315, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 337, 335, 330, 325, 320, 315, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (337, 100), point B can be (337, 95), point C can be (150, 95), and point D can be (150, 100).

Any substantially pure polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:97 over that length as determined herein is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 386, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 386, and a Y coordinate greater than or equal to 50; where point C has an X coordinate greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 386, 380, 375, 370, 375, 360, 365, 350, 325, 300, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 386, 380, 375, 370, 375, 360,

365, 350, 325, 300, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 350, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, 200, 300, 350, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (386, 100), point B can be (386, 95), point C can be (350, 95), and point D can be (350, 100).

Any method can be used to obtain a substantially pure polypeptide. For example, common polypeptide purification techniques such as affinity chromatography and HPLC as well as polypeptide synthesis techniques can be used. In addition, any material can be used as a source to obtain a substantially pure polypeptide. For example, tissue from wild-type or transgenic animals can be used as a source material. In addition, tissue culture cells engineered to over-express a particular polypeptide of interest can be used to obtain substantially pure polypeptide. Further, a polypeptide within the scope of the invention can be "engineered" to contain an amino acid sequence that allows the polypeptide to be captured onto an affinity matrix. For example, a tag such as c-myc, hemagglutinin, polyhistidine, or Flag™ tag (Kodak) can be used to aid polypeptide purification. Such tags can be inserted anywhere within the polypeptide including at either the carboxyl or amino termini. Other fusions that could be useful include enzymes that aid in the detection of the polypeptide, such as alkaline phosphatase.

The invention provides polypeptides that contain the entire amino acid sequence depicted in Figure 4, 9, 12, or 30. In addition, the invention provides polypeptides that contain a portion of the amino acid sequence depicted in Figure 4, 9, 12, or 30. For example, the invention provides polypeptides that contain a 15 amino acid sequence identical to any 15 amino acid sequence depicted in Figure 4, 9, 12, or 30 including, without limitation, the sequence starting at amino acid residue number 1 and ending at amino acid residue number 15, the sequence starting at amino acid residue number 2 and ending at amino acid residue number 16, the sequence starting at amino acid residue number 3 and ending at amino acid residue number 17, and so forth. It will be appreciated that the invention also provides polypeptides that contain an amino acid

sequence that is greater than 15 amino acid residues (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more amino acid residues) in length and identical to any portion of the sequence depicted in Figure 4, 9, 12, or 30. For example, the invention provides polypeptides that contain a 25 amino acid sequence identical to any 25 amino acid sequence depicted in Figure 4, 9, 12, or 30 including, without limitation, the sequence starting at amino acid residue number 1 and ending at amino acid residue number 25, the sequence starting at amino acid residue number 2 and ending at amino acid residue number 26, the sequence starting at amino acid residue number 3 and ending at amino acid residue number 27, and so forth. Additional examples include, without limitation, polypeptides that contain an amino acid sequence that is 50 or more amino acid residues (e.g., 100, 150, 200, 250, 300, 350, or more amino acid residues) in length and identical to any portion of the sequence depicted in Figure 4, 9, 12, or 30. Such polypeptides can include, without limitation, those polypeptides containing a amino acid sequence represented in a single line of sequence depicted in Figure 4, 9, 12, or 30 since each line of sequence depicted in these figures, with the possible exception of the last line, provides a 50 amino acid sequence.

In addition, the invention provides polypeptides that an amino acid sequence having a variation of the amino acid sequence depicted in Figure 4, 9, 12, or 30. For example, the invention provides polypeptides containing an amino acid sequence depicted in Figure 4, 9, 12, or 30 that contains a single insertion, a single deletion, a single substitution, multiple insertions, multiple deletions, multiple substitutions, or any combination thereof (e.g., single deletion together with multiple insertions). The invention provides multiple examples of polypeptides containing an amino acid sequence having a variation of an amino acid sequence depicted in Figure 4, 9, 12, or 30.

Figure 6 depicts the amino acid sequence depicted in Figure 4 (designated STdxsp) aligned with 20 other amino acid sequences. Examples of variations of the STdxsp sequence include, without limitation, any variation of the STdxsp sequence provided in Figure 6. Such variations are provided in Figure 6 in that a comparison of the amino acid residue (or lack thereof) at a particular position of the STdxsp sequence with the amino acid residue (or lack thereof) at the same position of any of the other 20 amino acid sequences depicted in Figure 6 provides a list of specific changes for the STdxsp

sequence. For example, the "t" at position 1148 of the STdxsp sequence can be substituted with an "s" as indicated in Figure 6. As also indicated in Figure 6, the "f" at position 575 of the STdxsp sequence can be substituted with an "m", "a", "l", "i", "y", or "v". For Figure 6, the nucleic acid numbering of Figure 2 is used to number the amino acid residue positions of the STdxsp sequence. Thus, the first amino acid residue of the STdxsp sequence starts with number 182 and proceeds in increments of three. It will be appreciated that the STdxsp sequence can contain any number of variations as well as any combination of types of variations. For example, the STdxsp sequence can contain one variation provided in Figure 6 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 6. It is noted that the 21 full-length amino acid sequences depicted in Figure 6 can be polypeptides having DXS activity.

Figure 14 depicts the amino acid sequence depicted in Figure 9 (designated RSddsp) and the amino acid sequence depicted in Figure 12 (designated STddsp) aligned with each other as well as aligned with three other amino acid sequences. For Figure 14, the nucleic acid numbering of Figure 7 is used to number the amino acid residue positions of the RSddsp sequence, and the nucleic acid numbering of Figure 10 is used to number the amino acid residue positions of the STddsp sequence. Thus, the first amino acid residue of the RSddsp and STddsp sequences each start with a number other than 1 and proceed in increments of three. Examples of variations of the RSddsp sequence include, without limitation, any variation of the RSddsp sequence provided in Figure 14. Examples of variations of the STddsp sequence include, without limitation, any variation of the STddsp sequence provided in Figure 14. Such variations are provided in Figure 14 in that a comparison of the amino acid residue (or lack thereof) at a particular position of the RSddsp sequence or the STddsp sequence with the amino acid residue (or lack thereof) at the same position of any of the other amino acid sequences depicted in Figure 14 provides a list of specific changes for the RSddsp sequence and the STddsp sequence. For example, the "l" at position 762 of the RSddsp sequence or the "l" at position 1007 of the STddsp sequence can be substituted with an "a" as indicated in Figure 14. Again, it will be appreciated that the RSddsp sequence as well as the STddsp sequence can contain any number of variations as well as any combination of types of variations. For example, the RSddsp sequence can contain one variation provided in Figure 14 or more than one

(e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 14. Likewise, the STddsp sequence can contain one variation provided in Figure 14 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 14. It is noted that the five full-length amino acid sequences depicted in Figure 14 can be polypeptides having DDS activity.

Figure 32 depicts the amino acid sequence depicted in Figure 30 (designated Stdxxrp) aligned with 15 other amino acid sequences. Examples of variations of the Stdxxrp sequence include, without limitation, any variation of the Stdxxrp sequence provided in Figure 32. Such variations are provided in Figure 32 in that a comparison of the amino acid residue (or lack thereof) at a particular position of the Stdxxrp sequence with the amino acid residue (or lack thereof) at the same position of any of the other 15 amino acid sequences depicted in Figure 32 provides a list of specific changes for the Stdxxrp sequence. It will be appreciated that the Stdxxrp sequence can contain any number of variations as well as any combination of types of variations. For example, the Stdxxrp sequence can contain one variation provided in Figure 32 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 32. It is noted that the full-length amino acid sequences depicted in Figure 32 can be polypeptides having DXR activity.

The invention also provides polypeptides containing an amino acid sequence that contains a variant of a portion of the amino acid sequence depicted in Figure 4, 9, 12, or 30 as described herein.

3. *Genetically modified cells*

Any cell containing an isolated nucleic acid within the scope of the invention is itself within the scope of the invention. This includes, without limitation, prokaryotic cells such as cells from the Rhodospirillaceae family (e.g., *Rhodobacter* cells) and eukaryotic cells such as plant and mammalian cells. It is noted that cells containing an isolated nucleic acid of the invention are not required to express the isolated nucleic acid. In addition, the isolated nucleic acid can be integrated into the genome of the cell or maintained in an episomal state. In other words, cells can be stably or transiently transformed with an isolated nucleic acid of the invention.

Any method can be used to introduce an isolated nucleic acid into a cell. In fact, many methods for introducing nucleic acid into a cell, whether *in vivo* or *in vitro*, are well known to those skilled in the art. For example, calcium phosphate precipitation, electroporation, heat shock, lipofection, microinjection, conjugation, and viral-mediated nucleic acid transfer are common methods that can be used to introduce nucleic acid into a cell. In addition, naked DNA can be delivered directly to cells *in vivo* as describe elsewhere (U.S. Patent Number 5,580,859 and U.S. Patent Number 5,589,466 including continuations thereof). Further, nucleic acid can be introduced into cells by generating transgenic animals.

Any method can be used to identify cells that contain an isolated nucleic acid within the scope of the invention. For example, PCR and nucleic acid hybridization techniques such as Northern and Southern analysis can be used. In some cases, immunohistochemistry and biochemical techniques can be used to determine if a cell contains a particular nucleic acid by detecting the expression of a polypeptide encoded by that particular nucleic acid. For example, detection of polypeptide X-immunoreactivity after introduction of an isolated nucleic acid containing a cDNA that encodes polypeptide X into a cell that does not normally express polypeptide X can indicate that that cell not only contains the introduced nucleic acid but also expresses the encoded polypeptide X from that introduced nucleic acid. In this case, the detection of any enzymatic activities of polypeptide X also can indicate that that cell contains the introduced nucleic acid and expresses the encoded polypeptide X from that introduced nucleic acid.

Any method can be used to direct the expression of an amino acid sequence from a nucleic acid. Such methods are well known to those skilled in the art, and include, without limitation, constructing a nucleic acid such that a regulatory element drives the expression of a nucleic acid sequence that encodes a polypeptide. Typically, regulatory elements are DNA sequences that regulate the expression of other DNA sequences at the level of transcription. Such regulatory elements include, without limitation, promoters, enhancers, and the like. In addition, any method for expressing a polypeptide from an exogenous nucleic acid molecule in microorganisms such as bacteria and yeast can be used. For example, well-known methods for making and using nucleic acid constructs that are capable of expressing exogenous polypeptides within *Rhodobacter* species (e.g.,

R. sphaeroides and *R. capsulatus*) can be used. See, e.g., Dryden and Dowhan, *J. Bacteriol.*, 178(4):1030-1038 (1996); Vasilyeva *et al.*, *Applied Biochemistry and Biotechnology*, 77-79:337-345 (1999); Graichen *et al.*, *J. Bacteriol.*, 181(14):4216-4222 (1999); Johnson *et al.*, *J. Bacteriol.*, 167(2):604-610 (1986); and Duport *et al.*, *Gene*, 5 145:103-108 (1994). Further, any methods can be used to identify cells that express an amino acid sequence from a nucleic acid. Such methods are well known to those skilled in the art, and include, without limitation, immunocytochemistry, Western analysis, Northern analysis, and RT-PCR.

The cells described herein can contain a single copy, or multiple copies (e.g., 10 about 5, 10, 20, 35, 50, 75, 100 or 150 copies), of a particular exogenous nucleic acid. For example, a bacterial cell can contain about 50 copies of exogenous nucleic acid X. In addition, the cells described herein can contain more than one particular exogenous nucleic acid. For example, a bacterial cell can contain about 50 copies of exogenous nucleic acid X as well as about 75 copies of exogenous nucleic acid Y. In these cases, 15 each different nucleic acid can encode a different polypeptide having its own unique enzymatic activity. For example, a bacterial cell can contain two different exogenous nucleic acids such that a high level of CoQ(10) is produced. In this example, such a cell can contain a first exogenous nucleic acid that encodes a polypeptide having DXS activity and a second exogenous nucleic acid that encodes a polypeptide having DDS activity. In 20 addition, a single exogenous nucleic acid can encode one or more than one polypeptide. For example, a single nucleic acid can contain sequences that encode three different polypeptides.

In addition to providing cells that contain an isolated nucleic acid of the invention, the invention provides cells (e.g., plant cells, animal cells, and microorganisms) that can 25 be used to produce an isoprenoid compound such as CoQ(10). The term "microorganism" as used herein refers to all microscopic organisms including, without limitation, bacteria, algae, fungi, and protozoa. It is noted that bacteria cells can be membraneous bacteria or non-membraneous bacteria.

The term "non-membraneous bacteria" as used herein refers to any bacteria 30 lacking intracytoplasmic membrane. The term "membraneous bacteria" as used herein refers to any naturally-occurring, genetically modified, or environmentally modified

- bacteria having an intracytoplasmic membrane. An intracytoplasmic membrane can be organized in a variety of ways including, without limitation, vesicles, tubules, thylakoid-like membrane sacs, and highly organized membrane stacks. Any method can be used to analyze bacteria for the presence of intracytoplasmic membranes including, without
- 5 limitation, electron microscopy, light microscopy, and density gradients. *See, e.g., Chory et al., J. Bacteriol.*, 159:540-554 (1984); Niederman and Gibson, Isolation and Physiochemical Properties of Membranes from Purple Photosynthetic Bacteria. In: The Photosynthetic Bacteria, Ed. By Roderick K. Clayton and William R. Sistrom, Plenum Press, pp. 79-118 (1978); and Lueking *et al., J. Biol. Chem.*, 253: 451-457 (1978).
- 10 Examples of membraneous bacteria that can be used herein include, without limitation, bacteria of the Rhodospirillaceae family such as those in the genus Rhodobacter (e.g., *R. sphaeroides*, *R. capsulatus*, *R. sulfidophilus*, *R. adriaticus*, and *R. veldkampii*), the genus Rhodospirillum (e.g., *R. rubrum*, *R. photometricum*, *R. molischianum*, *R. fulvum*, and *R. salinarum*), the genus Rhodopseudomonas (e.g., *R. palustris*, *R. viridis*, and *R.*
- 15 *sulfovirens*), the genus Rhodomicrobium, the genus Rhodocyclus, and the genus Rhodopila; bacteria of the Chromatiaceae family such as those in the genus Chromatium, genus Thiocystis, the genus Thiospirillum, the genus Thiocapsa, the genus Lamprobacter, the genus Lalmprocystis, the genus Thiodictyon, the genus Amoebobacter, and the genus Thiopedia; green sulfur bacteria such as those in the genus Chlorobium and the genus
- 20 Prosthecochloris; bacteria of the Methylococcaceae family such as those in the genus Methylococcus (e.g., *M. capsulatus*), and the genus Methylomonas (e.g., *M. methanica*); and particular bacteria of the Nitrobacteraceae family such as those in the genus Nitrobacter (e.g., *N. winogradsky* and *N. hamburgensis*), the genus Nitrococcus (e.g., *N. mobilis*), and the genus Nitrosomonas (e.g., *N. europaea*).
- 25 Membraneous bacteria can be highly membraneous bacteria. The term "highly membraneous bacteria" as used herein refers to any bacterium having more intracytoplasmic membrane than *R. sphaeroides* (ATCC 17023) cells have after the *R. sphaeroides* (ATCC 17023) cells have been (1) cultured chemoheterotrophically under aerobic conditions for four days, (2) cultured chemoheterotrophically under oxygen-
- 30 limited conditions for four hours, and (3) harvested. The aerobic culture conditions involve culturing the cells in the dark at 30°C in the presence of 25 percent oxygen. The

oxygen-limited conditions involve culturing the cells in the light at 30°C in the presence of 2 percent oxygen. After the four hour culturing step under oxygen-limited conditions, the *R. sphaeroides* (ATCC 17023) cells are harvested by centrifugation and analyzed.

Typically, any cell (e.g., membranous bacteria) can be genetically modified such that a particular isoprenoid compound is produced. Such cells can contain exogenous nucleic acid that encodes a polypeptide having enzymatic activity. For example, a microorganism having endogenous DDS activity can be transformed with an exogenous nucleic acid that encodes a polypeptide having DDS activity. In this case, the microorganism can have increased DDS activity which can lead to an increased production of CoQ(10). Thus, a cell can be given an exogenous nucleic acid that encodes a polypeptide having an enzymatic activity that catalyzes the production of a compound normally produced by that cell. In this case, the genetically modified cell can produce more of the compound, or can produce the compound more efficiently, than a similar cell not having the genetic modification. Alternatively, a cell can be given an exogenous nucleic acid that encodes a polypeptide having an enzymatic activity that catalyzes the production of a compound that is not normally produced by that cell.

The invention provides cells containing exogenous nucleic acid that encodes a polypeptide having enzymatic activity that leads to an increased production of CoQ(10). Such cells can contain nucleic acid that encodes a polypeptide having DDS activity. Other examples include, without limitation, cells containing exogenous nucleic acid that encodes polypeptides having DXS, ODS, SDS, DXR, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase (e.g., *ispD*), 4-diphosphocytidyl-2C-methyl-D-erythritol kinase (e.g., *ispE*), and/or chorismate lyase (e.g., *ubiC*) activity. Nucleic acid molecules that encode polypeptides having such enzymatic activities can be obtained as described herein. For example, nucleic acid encoding a polypeptide having chorismate lyase can be cloned using the sequence information provided in Genbank[®] accession number X66619.

Typically, microorganisms of the invention produce CoQ(10) with the yield (mg of CoQ(10) per g of dry biomass) being at least about 5 (e.g., at least about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, or more) percent greater than that of a comparable wild-type strain grown under similar conditions. Bacteria can produce more CoQ(10) when grown under anaerobic conditions as compared to aerobic conditions. For example,

anaerobically cultured bacteria can produce about 3 to 4 fold more CoQ(10) than aerobically cultured bacteria of the same species. When determining the yield of isoprenoid compound production for a particular cell (e.g., microorganism), any method can be used. See, e.g., Cohen-Bazire *et al.*, *J. Cell Comp. Physiol.*, 49:25-68 (1957);
5 Edlund, *J. Chromatogr.*, 425:87-97 (1988); Rousseau and Varin, *J. Chromatogr. Sci.*, 36:247-52 (1998); and Leray *et al.*, *J. Lipid Res.*, 39:2099-2105 (1998).

The invention provides a cell containing an exogenous nucleic acid that encodes a polypeptide having DXS, DDS, ODS, SDS, DXR, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase (e.g., ispD), 4-diphosphocytidyl-2C-methyl-D-erythritol kinase (e.g.,
10 ispE), and/or chorismate lyase (e.g., ubiC) activity. Nucleic acid molecules that encode polypeptides having such enzymatic activities can be obtained as described herein. The invention also provides a cell that contains more than one different exogenous nucleic acid molecule with each different exogenous nucleic acid molecule encoding a polypeptide having a different one of the following enzymatic activities: DXS, DDS,
15 ODS, SDS, DXR, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase (e.g., ispD), 4-diphosphocytidyl-2C-methyl-D-erythritol kinase (e.g., ispE), and/or chorismate lyase (e.g., ubiC) activity. For example, the invention provides a cell containing a first exogenous nucleic acid encoding a polypeptide having DXS activity and a second exogenous nucleic acid encoding a polypeptide having DDS activity.

20 The invention provides a cell containing an exogenous nucleic acid containing a dxs sequence (e.g., Stdxs sequence), dds sequence (e.g., StdDs or Rsdds sequence), dxr sequence (e.g., Stdxr sequence), ubiC sequence (e.g., EcUbiC sequence), or lytB sequence (e.g., RsLytB sequence). Such nucleic acids can be obtained as described herein. The invention also provides a cell that contains more than one of the following
25 sequences: a dxs sequence (e.g., Stdxs sequence), dds sequence (e.g., StdDs or Rsdds sequence), dxr sequence (e.g., Stdxr sequence), ubiC sequence (e.g., EcUbiC sequence), or lytB sequence (e.g., RsLytB sequence). For example, the invention provides a cell containing a first exogenous nucleic acid containing a dds sequence and a second exogenous nucleic acid containing a dxs sequence. Likewise, the invention provides a
30 cell containing a single exogenous nucleic acid that contains a dds sequence and a dxs sequence.

Typically, a microorganism within the scope of the invention catabolizes a hexose carbon such as glucose. A microorganism, however, can catabolize a pentose carbon (e.g., ribose, arabinose, xylose, and lyxose). In other words, a microorganism within the scope of the invention can either utilize hexose or pentose carbon. In addition, a
5 microorganism within the scope of the invention can use carbon sources such as methanol and/or organic acids (e.g., succinic acid or malic acid).

Any cells described herein can have reduced enzymatic activity such as reduced geranylgeranyl pyrophosphate synthase and/or magnesium protoporphyrin IX chelatase activity. Any cell described herein can have reduced biological activity such as reduced
10 activity of aerobic repressor polypeptides (e.g., PPSR) or oxidation-reduction sensor polypeptides (e.g., CBB3). In the case of multi-subunit molecules such as CBB3, the activity of the oxidation-reduction sensor polypeptide can be reduced by inactivating one or more than one of the subunits. For example, CBB3 activity can be reduced by inactivating a single subunit of CBB3 such as the ccoN subunit.

15 The term “reduced” as used herein with respect to a cell and a particular activity (e.g., particular enzymatic activity) refers to a lower level of activity than that measured in a comparable cell of the same species. Thus, a *R. sphaeroides* cell lacking geranylgeranyl pyrophosphate synthase activity is considered to have reduced geranylgeranyl pyrophosphate synthase activity since most, if not all, comparable *R.*
20 *sphaeroides* cells have at least some geranylgeranyl pyrophosphate synthase activity. Such reduced enzymatic activities can be the result of lower enzyme concentration, lower specific activity of an enzyme, or combinations thereof.

Many different methods can be used to make a cell having reduced enzymatic and/or biological activity. For example, a *R. sphaeroides* cell can be engineered to have a
25 disrupted enzyme-encoding locus using common mutagenesis or knock-out technology. Alternatively, antisense technology can be used to reduce enzymatic activity. For example, a *R. sphaeroides* cell can be engineered to contain a cDNA that encodes an antisense molecule that prevents an enzyme from being made. The term “antisense molecule” as used herein encompasses any nucleic acid that contains sequences that
30 correspond to the coding strand of an endogenous polypeptide. An antisense molecule also can have flanking sequences (e.g., regulatory sequences). Thus, antisense molecules

can be ribozymes or antisense oligonucleotides. A ribozyme can have any general structure including, without limitation, hairpin, hammerhead, or axhead structures, provided the molecule cleaves RNA.

Cells having a reduced enzymatic and/or biological activity can be identified using any method. For example, a *R. sphaeroides* cell having reduced geranylgeranyl pyrophosphate synthase activity can be easily identified using common biochemical methods that measure geranylgeranyl pyrophosphate synthase activity. See, e.g., Math *et al.*, *Proc. Natl. Acad. Sci. USA*, 89(15):6761-6764 (1992).

The invention provides a cell containing reduced geranylgeranyl diphosphate synthase, aerobic repressor, and/or cbb3-type cytochrome oxidase activity. Such cells can have reduced geranylgeranyl diphosphate synthase, aerobic repressor, and/or cbb3-type cytochrome oxidase activity as a result of disrupting the endogenous sequences that encode polypeptides having these activities. For example, a cell can have reduced geranylgeranyl diphosphate synthase activity as a result of knocking out a portion of the endogenous crtE sequence within a cell's genome; a cell can have reduced aerobic repressor activity as a result of knocking out a portion of the endogenous ppsR sequence within a cell's genome; and a cell can have reduced cbb3-type cytochrome oxidase activity as a result of knocking out a portion of the endogenous ccoN sequence within a cell's genome.

The invention also provides a cell containing non-functional crtE, ppsR, and/or ccoN nucleic acid sequences within its genome such that the encoded polypeptide is either mutated or not expressed. Such cells can be used to produce large amounts of CoQ(10). The sequence of crtE can be as set forth in Genbank® accession number AJ010302. The sequence of ppsR can be as set forth in Genbank® accession number AJ010302 or L19596. The sequence of ccoN can be as set forth in Genbank® accession number U58092. Knockout technology can be used to make cells containing non-functional crtE, ppsR, and/or ccoN nucleic acid sequences.

4. Producing isoprenoid compounds

The cells described herein can be used to produce isoprenoid compounds. For example, a microorganism having endogenous DDS activity can be transformed with

nucleic acid that encodes a polypeptide having DDS activity such that the microorganism produces more CoQ(10) than had the microorganism not been given that nucleic acid. Once transformed, the microorganism can be used cultured under conditions optimal for CoQ(10) production.

5 In addition, substantially pure polypeptides having enzymatic activity can be used alone or in combination with cells to produce isoprenoid compounds. For example, a preparation containing a substantially pure polypeptide having DDS activity can be used to catalyze the formation of CoQ(10). Further, cell-free extracts containing a polypeptide having enzymatic activity can be used alone or in combination with substantially pure
10 polypeptides and/or cells to produce isoprenoid compounds. For example, a cell-free extract containing a polypeptide having DXS activity can be used to form 1-deoxyxyulose-5-phosphate, while a microorganism containing polypeptides have the enzymatic activities necessary to catalyze the reactions needed to form CoQ(10) from 1-deoxyxyulose-5-phosphate can be used to produce CoQ(10). Any method can be used to
15 produce a cell-free extract. For example, osmotic shock, sonication, and/or a repeated freeze-thaw cycle followed by filtration and/or centrifugation can be used to produce a cell-free extract from intact cells.

 It is noted that a cell, substantially pure polypeptide, and/or cell-free extract can be used to produce a particular isoprenoid compound that is, in turn, treated chemically to
20 produce another compound. For example, a microorganism can be used to produce CoQ(10), while a chemical process is used to modify CoQ(10) into a CoQ(10) derivative such as CoQ10 containing a polar group. Likewise, a chemical process can be used to produce a particular compound that is, in turn, converted into an isoprenoid compound using a cell, substantially pure polypeptide, and/or cell-free extract described herein. For
25 example, a chemical process can be used to produce deoxyxyulose-5-phosphate, while a microorganism can be used convert deoxyxyulose-5-phosphate into CoQ(10).

 Typically, a particular isoprenoid compound is produced by providing a microorganism and culturing the provided microorganism with culture medium such that that isoprenoid compound is produced. In general, the culture media and/or culture
30 conditions can be such that the microorganisms grow to an adequate density and produce the desired compound efficiently. For large-scale production processes, the following

methods can be used. First, a large tank (e.g., a 100 gallon, 200 gallon, 500 gallon, or more tank) containing appropriate culture medium with, for example, a glucose carbon source is inoculated with a particular microorganism. After inoculation, the microorganisms are incubated to allow biomass to be produced. Once a desired biomass is reached, the broth containing the microorganisms can be transferred to a second tank. This second tank can be any size. For example, the second tank can be larger, smaller, or the same size as the first tank. Typically, the second tank is larger than the first such that additional culture medium can be added to the broth from the first tank. In addition, the culture medium within this second tank can be the same as, or different from, that used in the first tank. For example, the first tank can contain medium with xylose, while the second tank contains medium with glucose.

Once transferred, the microorganisms can be incubated to allow for the production of the desired isoprenoid compound. Once produced, any method can be used to isolate the desired compound. For example, if the microorganism releases the desired isoprenoid compound into the broth, then common separation techniques can be used to remove the biomass from the broth, and common isolation procedures (e.g., extraction, distillation, and ion-exchange procedures) can be used to obtain the isoprenoid compound from the microorganism-free broth. In addition, the desired isoprenoid compound can be isolated while it is being produced, or it can be isolated from the broth after the product production phase has been terminated. If the microorganism retains the desired isoprenoid compound, then the biomass can be collected and treated to release the isoprenoid compound, and the released isoprenoid compound can be isolated.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

25

EXAMPLES

Example 1 – Cloning nucleic acid that encodes a *Sphingomonas trueperi* polypeptide having DXS activity

S. trueperi cells were obtained from the American Type Culture Collection (ATCC Cat. No. 12417). To isolate bacterial genomic DNA, cells were grown in 100-200 mL cultures for 2-3 days at 30°C on a shaker rotating at 250 rpm. Cultured cells

30

were centrifuged to form a cell pellet, washed by resuspending the pellet in a solution of 10 mM Tris/1 mM EDTA, and centrifuged again as before. The cell pellets were resuspended in 5 mL of GTE buffer per 100 mL of original culture. GTE buffer is 50 mM glucose/25 mM Tris-HCl (pH 8.0)/10 mM EDTA (pH 8.0). The bacterial cell walls were lysed by adding lysozyme (final concentration of 1 mg/mL), Proteinase K (final concentration of 1 mg/mL), and mutanolysin (final concentration of 5.5 µg/mL) to the resuspended cell solution to form a lysing mixture that was incubated for 90 minutes at 37°C. After this incubation, sodium dodecyl sulfate was added to the mixture to a final concentration of 1 percent, and additional Proteinase K was added until the concentration in the solution was 2 mg/mL. After a 1 hour incubation at 50°C, the solution containing the lysed cells was diluted 1:1 with fresh GTE buffer. Once diluted, sodium chloride was added to the solution to a final concentration of 0.15 M. Polypeptides and molecules other than nucleic acids were removed from the lysed bacterial cell solution by adding an equal volume of an organic mixture made up of phenol, chloroform, and isoamyl alcohol at a ratio of 25:24:1 (hereinafter referred to as PCIA). After adding PCIA, the solution was mixed. To separate the organic phase from the DNA-containing aqueous phase, the mixture was centrifuged at 12,000 x g for 10 minutes. The aqueous phase was transferred to a clean tube and re-extracted with an equal volume of chloroform alone. The aqueous and organic phases were separated by centrifugation at 3,000 x g for 10 minutes. The aqueous phase was again removed to a new tube and treated with 2.5 mg of RNase to degrade any bacterial RNA present. The purified DNA was recovered by adding 2.5 volumes of ethanol to the aqueous phase. After mixing the solution, the precipitated DNA was removed by spooling it on a glass rod. The spooled DNA was rinsed with 70 percent ethanol. Once rinsed, the ethanol was allowed to evaporate by leaving the DNA exposed to the air until dry. The dried DNA was resuspended in a solution of 10 mM Tris (pH 8.5). The resuspended DNA was re-extracted with PCIA followed by chloroform alone as before. The DNA was re-precipitated by adding one-tenth volume of 7.5 M ammonium acetate and 2.5 volumes ethanol, followed by spooling, rinsing, and air drying. The purified DNA was resuspended in 10 mM Tris (pH 8.5).

The following polymerase chain reaction (PCR) procedure was used to isolate nucleic acid that encodes a *S. trueperi* polypeptide having DXS activity. Three

degenerate forward PCR primers (F1, F2, and F3) and three degenerate reverse PCR primers (R1, R2, and R3) were designed by comparing sequences of several clones that encode polypeptides have DXS activity (Figure 15). The sequence of each degenerate primer was as follows:

- 5 F1: 5'-RTKATTYTM AAYGAY AAYGAAATG-3' (SEQ ID NO:53)
 F2: 5'-TTTGAAGARYTVGGYWTTAACTA-3' (SEQ ID NO:54)
 F3: 5'-RCAYCARGCTTAYSCVCAYAA-3' (SEQ ID NO:55)
 R1: 5'-CGTGYTGYTCDGCRATHGCBAC-3' (SEQ ID NO:56)
 R2: 5'-TGYTCDGCRATHGCBACRTCRAA-3' (SEQ ID NO:57)
 10 R3: 5'-GGSCCDATRTAGTTAAWRCC-3' (SEQ ID NO:58)

The primers were used in all logical combinations in PCR using Taq polymerase (Roche Molecular Biochemicals, Indianapolis, IN) and 1 ng of purified genomic DNA per microliter of reaction mix. Each PCR reaction was conducted using a touchdown PCR
 15 program with four cycles at each of the following annealing temperatures: 60°C, 58°C, 56°C, and 54°C, followed by 25 cycles at 52°C. Each cycle had an initial 30 second denaturing step at 94°C and a 90 second extension step at 72°C. The program had an initial denaturing step of 2 minutes at 94°C and final extension step of 5 minutes at 72°C.

Between about 2 µM and 12 µM of each PCR primer was used in each reaction,
 20 depending on the degree of degeneracy. After each PCR reaction was complete, a portion of each reaction was separated by gel electrophoresis using a 1.5 percent TAE (Tris-acetate-EDTA) agarose gel. The results from the gel electrophoresis indicated that the combination of degenerate primer F3 with degenerate primer R2 produced a nucleic acid molecule of 882 bp (referred to as the F3R2 fragment). The F3R2 fragment was purified
 25 away from the agarose gel matrix using the Qiagen Gel Extraction procedure according to the manufacturer's instructions (Qiagen Inc., Valencia, CA). A portion of the purified fragment was ligated into the pCRII-TOPO vector. The vector containing the F3R2 fragment was inserted into *E. coli* TOP10 cells using the TOPO cloning procedure (Invitrogen, Carlsbad, CA). The transformed TOP10 cells were plated onto LB agar
 30 plates containing 100 µg/mL of ampicillin (Amp) and 50 µg/mL of 5-Bromo-4-Chloro-3-Indolyl-β-D-Galactopyranoside (Xgal). Single white colonies were re-plated onto fresh

LB-Amp-Xgal plates and screened by PCR with the F3 and R2 primers to confirm the presence of plasmids with the desired insert. Plasmid DNAs were obtained from bacterial colonies using the QiaPrep Spin Miniprep Kit (Qiagen, Inc). The plasmid DNAs were then quantified and sequenced with the M13 forward and reverse primers. Sequence
5 analysis indicated that the sequence of the F3R2 fragment aligned with sequences from other nucleic acid molecules that encode polypeptides having DXS activity.

To obtain the complete coding sequence for the *S. trueperi* polypeptide having DXS activity, genome walking was performed as follows. Primers were designed based upon the sequence of the 882 bp F3R2 fragment for walking in both the upstream and
10 downstream directions. These walking primers had the following sequences:

GSP1F: 5'-TCGTGACCAAGAAGGGCAAGGGCTATG-3'(SEQ ID NO:59)
GSP2F: 5'-GACAAGTATCACGGCGTCCAGAAGTTC-3' (SEQ ID NO:60)
GSP1R: 5'-ATAGCCCTTGCCCTTCTTGGTCACGAC-3' (SEQ ID NO:61)
15 GSP2R: 5'-CGAACGGATCATACTCGCTCTCGCTG-3' (SEQ ID NO:62)

The GSP1F and GSP2F primers are primers that face downstream of the DXS polypeptide start codon, while the GSP1R and GSP2R primers are primers that face in the opposite direction. In addition, GSP2F and GSP2R are nested inside of the GSP1F and
20 GSP1R primers. Genome walking was conducted according to the manual of CLONTECH's Universal Genome Walking kit (CLONTECH Laboratories, Inc., Palo Alto, CA) with the exception that *Fsp* I and *Sma* I were used instead of *Dra* I and *EcoR* V. The genomic DNA used was from *S. trueperi*. DMSO was added to the PCR mixture until a final concentration of 5 percent was reached. The PCR reactions were performed
25 using a Perkin Elmer 9700 Thermocycler. The first round of PCR consisted of 7 cycles of 2 seconds at 94°C and 3 minutes at 72°C, followed by 36 cycles of 2 seconds at 94°C and 3 minutes at 67°C, with a final extension at 67°C for 4 minutes. The second round of PCR consisted of 5 cycles of 2 seconds at 94°C and 3 minutes at 72°C, followed by 24
30 cycles of 2 seconds at 94°C and 3 minutes at 67°C, with a final extension at 67°C for 4 minutes. After the PCR was complete, a portion of the reaction mix from each round was separated by gel electrophoresis using a 1.5 percent TAE agarose gel. Good

amplification products were obtained with the *Pvu* II and *Stu* I libraries using the GSP1F and GSP2F primers and with the *Fsp* I and *Pvu* II libraries using the GSP1R and GSP2R primers. The second round products from each of these libraries were gel purified, cloned using the TOPO cloning procedure (Invitrogen, Carlsbad, CA), and sequenced. A 1.7
5 kilobase (kb) fragment was subcloned from the *Pvu* IIF library, a 2.8 kb fragment was subcloned from the *Stu* IF library, a 400 bp fragment was subcloned from the *Fsp* IR library, and a 330 bp fragment was subcloned from the *Pvu* IIR library. Each of these subcloned fragments was sequenced. Sequence analysis indicated that each subcloned
10 fragment contained a sequence that overlapped with that of the F3R2 fragment and was similar to other nucleic acid sequences that encode polypeptides having DXS activity.

Because the sequence information obtained by genome walking extended 13 bp upstream of the translational start codon, a second genome walk was conducted to gain additional sequence information. This second walk used GSPB2R, 5'-TGAGGATCTTGTGCGGATAGC-ATTGGTG-3' (SEQ ID NO:63) as the first round
15 primer and GSPB3R, 5'-AGCGGCGTCTTG-GGTAGGTCAGCCAT-3' (SEQ ID NO:64) as the second round primer. The second walk was conducted using only the *Sma* I and *Stu* I libraries. CLONTECH's Advantage-GC Genomic Polymerase was used for PCR with a 1.0 mM GC Melt concentration according to the manufacturer's specifications. The first round of PCR was conducted using a Perkin Elmer 9700
20 Thermocycler with an initial denaturing step at 96°C for 5 seconds followed by 7 cycles consisting of 2 seconds at 94°C and 3 minutes at 72°C, followed by 36 cycles consisting of 2 seconds at 94°C and 3 minutes at 66°C, with a final extension at 66°C for 4 minutes. The second round of PCR had 5 cycles consisting of 2 seconds at 94°C and 3 minutes at 72°C, followed by 26 cycles consisting of 2 seconds at 94°C and 3 minutes at 66°C, with
25 a final extension at 66°C for 4 minutes. Portions of the PCR products from each round were separated by gel electrophoresis using a 1.5 percent TAE agarose gel. The gel electrophoresis revealed the presence of a 250 bp amplification product obtained from the second round of PCR using the *Stu* I library. This fragment was gel purified, cloned using the TOPO cloning procedure (Invitrogen, Carlsbad, CA), and sequenced. An
30 overlap with the previously obtained sequence was found, extending the length of the clone to 181 bp before the start codon. The full-length clone containing coding and non-

coding sequence was 3626 bp in length (Figure 2). The open reading frame was 1926 bp in length (Figure 3), which encoded a polypeptide with 641 amino acid residues (Figure 4).

The coding sequence of the DXS polypeptide was amplified by PCR using *S. trueperi* genomic DNA as template. Primers were designed based on the sequence obtained above. The sequences of the primers were as follows:

SHDXF1: 5'-ATATGGTACCGTGTGACTGACCTGTCCAAC-3' (SEQ ID NO:65)

SHDXR1: 5'-AGTCTCTAGAAATGTTGGAGATTCAAGGTGG-3' (SEQ ID NO:66)

10

These primers were designed to introduce a *Kpn* I restriction site at the beginning of the amplified fragment and an *Xba* I restriction site at the end of the amplified fragment. The sequence of each restriction site is underlined. The PCR reaction mix contained the following: 100 ng genomic DNA, 2 μ L of each primer (SHDXF1 and SHDXR1, each at 15 50 μ M), 10 μ L 10X *Pfu* Plus buffer, 5 μ L DMSO, 8 μ L dNTPs (10 μ M each) and 5 units *Pfu* polymerase in a final volume of 100 μ L. Each PCR reaction was performed in a Perkin Elmer Geneamp PCR system 2400 under the following conditions: an initial denaturation at 94°C for 5 minutes; 8 cycles of (1) 94°C for 45 seconds, (2) 55°C for 45 seconds, and (3) 72°C for 3 minutes; 21 cycles of (1) 94°C for 45 seconds, (2) 61°C for 20 45 seconds and (3) 72°C for 3 minutes; and a final extension of 72°C for 10 minutes. A portion of the PCR reaction was separated by gel electrophoresis using a 0.8 percent TAE gel. The gel electrophoresis revealed a 1.6 kb fragment. This fragment was (1) purified using a Qiagen Gel Extraction kit (Qiagen Inc., Valencia, CA), (2) treated with *Kpn* I and *Xba* I (New England BioLabs, Inc., Beverly, MA), and (3) subcloned into 25 pUC18 that had also been treated with *Kpn* I and *Xba* I and gel purified. The resulting construct designated appUC18-SHDXS is depicted in Figure 18. The ligation was carried out with T4 DNA ligase at 16°C for 16 hours. Once ligated, 1 μ L was used to electroporate *E. coli* ElectroMAX™ DH10B™ cells (Life Technologies, Inc., Rockville, MD). The electroporated cells were plated on LB-Amp plates (Amp concentration = 100 30 μ g/mL). From these plates, eight individual colonies were chosen at random. The plasmid was isolated from each colony using a QiaPrep Spin Miniprep kit (Qiagen Inc.,

Valencia, CA). The extracted plasmid DNA was examined for the presence of the 1.6 kb fragment by digesting individual aliquots with one of three different restriction enzymes: *EcoR* I, *BamH* I, and *Nar* I. If the plasmids contained the correct 1.6 kb fragment, the *EcoR* I digest reaction would result in two fragments (0.77 and 4.13 kb), the *BamH* I digest reaction would result in one fragment (4.8 kb), and the *Nar* I digest reaction would result in two fragments (1.9 and 2.9 kb). After treating with the restriction enzymes, the digest reactions were separated by gel electrophoresis using a 0.8 percent TAE agarose gel. All 8 clones yielded digestion fragments consistent with a clone of 1.6 kb.

10 Example 2 – Introducing nucleic acid that
 encodes a polypeptide having DXS activity into cells

The nucleic acid molecule that encodes a polypeptide having DXS activity and was obtained as described in Example 1 is introduced into cells as follows. First, a construct is made to contain the nucleic acid molecule such that the encoded polypeptide having DXS activity is expressed in a desired host cell. When using prokaryotic cells, a construct functional in prokaryotic cells is used. When using eukaryotic cells, a construct functional in eukaryotic cells is used. Second, the construct is introduced into the desired host cell using appropriate methods. Once introduced, stable transformants are selected.

20 Example 3 – Cloning nucleic acid that encodes
 a *Rhodobacter sphaeroides* polypeptide having DDS activity

R. sphaeroides ATCC strain 17023 cells were grown in 550 R 8 A H media at 30°C and 100 rpm. The recipe for 550 R 8 A H media was provided by ATCC. Genomic DNA was isolated from *R. sphaeroides* cells as described in Example 1.

25 To isolate nucleic acid encoding an *R. sphaeroides* polypeptide having DDS activity, degenerate primers were designed and used as described in Example 1. Briefly, three degenerate forward primers (F4, F5, and F6) and four degenerate reverse primers (R4, R5, R6, and R7) were designed by comparing sequences of several clones that encode polypeptides have DDS, SDS, or ODS activity (Figure 16). The sequence of each
30 degenerate primer was as follows:

- F4: 5'-GGWGGHAARMGMMTKCGYCC-3' (SEQ ID NO:67)
F5: 5'-ACWYTGSTDCATGATGATGT-3' (SEQ ID NO:68)
F6: 5'-ACNYTNBTNCAYGAYGAYGT-3' (SEQ ID NO:69)
R4: 5'-TYRTCACATCATCATG-3' (SEQ ID NO:70)
5 R5: 5'-TGHAVKACYTCACCYTCRGMAAT-3' (SEQ ID NO:71)
R6: 5'-TARTCNARDATRTCTCDAT-3' (SEQ ID NO:72)
R7: 5'-TCRTCNCNAYNKTYTTNCC-3' (SEQ ID NO:73)

These primers were used in all logical combinations in PCR using Taq polymerase (Roche Molecular Biochemicals, Indianapolis, IN) and 1 ng of genomic DNA per microliter of reaction mix. PCR was conducted using the touchdown PCR program as described in Example 1. Between about 4 μ M and 8 μ M of each PCR primer was used in each reaction, depending on the degree of degeneracy. After each PCR reaction was complete, a portion of each reaction was separated by gel electrophoresis using a 1.5 percent TAE agarose gel. The results from the gel electrophoresis yielded no fragments of the expected size. A second amplification reaction was then performed using each sample from the first round of PCR. Briefly, one μ L of reaction mixture from each first round of PCR was used in a 50 μ L amplification reaction using the same primer pairs and thermocycling parameters used in the first round of PCR. A portion of each of the second round PCR reactions was separated by gel electrophoresis using a 1.5 percent TAE agarose gel. The combination of degenerate primers F6 and R5 produced a fragment of 209 bp (referred to as the F6R5 fragment). The F6R5 fragment was isolated from an agarose gel and purified using the Qiagen Gel Extraction procedure (Qiagen Inc., Valencia, CA). An aliquot of the purified fragment was ligated to pCRII-TOPO, and the product of the ligation reaction was inserted into TOP10 *E. coli* cells using a TOPO cloning procedure (Invitrogen, Carlsbad, CA). The products of the individual insertion reactions were plated onto LB media containing 100 μ g/mL Amp and 50 μ g/mL Xgal. Single white colonies that grew on the LB-Amp-Xgal plates were re-plated onto fresh LB-Amp plates and screened in a PCR reaction using the F6 and R5 primers to confirm the presence of the desired insert. Plasmid DNAs were obtained from several colonies using a QiaPrep Spin Miniprep kit (Qiagen, Inc). The obtained plasmid DNAs were

quantified and sequenced with the M13 forward and reverse primers. Sequence analysis revealed that the F6R5 fragment contained sequences that aligned with sequences from other nucleic acid molecules that encode polypeptides having polyprenyl diphosphate synthase activity.

5 Genome walking was performed to obtain a complete coding sequence for the *R. sphaeroides* DDS polypeptide using procedures similar to those described in Example 1. Briefly, primers were designed based on the sequence of the F6R5 fragment for walking in both the upstream and downstream directions. These primers had the following sequences:

10

GSP3F: 5'-TGGAAGCTGCGGGCGAAGAGATAGTC-3' (SEQ ID NO:74)

GSP4F: 5'-CCCACCAGCACCGAGGATTTGTTGTC-3' (SEQ ID NO:75)

GSP3R: 5'-GAACCTGCTGTGGGACAACAAATCCTC-3' (SEQ ID NO:76)

GSP4R: 5'-TCGGTGCTGGTGGGCGACTATCTCTTC-3' (SEQ ID NO:77)

15

The GSP3F and GSP4F primers are primers that face downstream of the DDS polypeptide start codon, while the GSP3R and GSP4R primers are primers that face in the opposite direction. In addition, the GSP4F and GSP4R primers are nested inside the GSP3F and GSP3R primers.

20 The *Pvu* II, *Fsp* I, and *Stu* I libraries with the GSP3F and GSP4F primers and all four libraries with the GSP3R and GSP4R primers resulted in the production of amplified fragments. A 750 bp fragment from the *Pvu* I library, a 500 bp fragment from the *Fsp* I library, a 1.4 kb fragment from the *Stu* I library, and a 0.9 kb fragment from the *Sma* I library were all subcloned and sequenced. Sequence analysis indicated that each
25 subcloned fragment contained a sequence that overlapped with the sequence of the F6R5 fragment and was similar to other nucleic acid sequences that encode polypeptides having polyprenyl diphosphate synthase activity. The full-length clone containing coding and non-coding sequence was 1990 bp in length (Figure 7). The open reading frame was 1002 bp in length (Figure 8), which encoded a polypeptide with 333 amino acid residues
30 (Figure 9).

The coding sequence of the DDS polypeptide from *R. sphaeroides* was amplified by PCR using *R. sphaeroides* genomic DNA as template. PCR primers were designed based on the sequences obtained as described above. The sequences of the primers were as follows.

5

RDS18F: 5'-ACTAGAAATTCCGCAACAGTTCCTTCATGTC-3' (SEQ ID NO:78)

RDS18R: 5'-ATAGAAAGCTTACTTGCGGTCGGACTGATAG-3' (SEQ ID NO:79)

10 These primers were designed to introduce an *EcoR* I restriction site at the beginning of the amplified fragment and a *Hind* III restriction site at the end of the amplified fragment. The sequence of each restriction site is underlined. The PCR reaction mix contained the following: 100 ng genomic DNA, 2 µL of each primer (RDS18F and RDS18R, each at 50 µM), 10 µL 10X *Pfu* Plus buffer, 5 µL DMSO, 8 µL dNTPs (10 mM each) and 5 units *Pfu* polymerase in a final volume of 100 µL. Each PCR reaction was performed in a Perkin
15 Elmer Geneamp PCR system 2400 under the following conditions: an initial denaturation at 94°C for 5 minutes; 8 cycles of (1) 94°C for 45 seconds, (2) 55°C for 45 seconds, and (3) 72°C for 3 minutes; 21 Cycles of (1) 94°C for 45 seconds, (2) 61°C for 45 seconds, and (3) 72°C for 3 minutes; and a final extension of 72°C for 10 minutes. After completing the PCR reactions, each PCR reaction was separated by gel electrophoresis
20 using a 0.8 percent TAE agarose gel. The gel electrophoresis revealed a 1.6 kb fragment. This fragment was (1) purified from the agarose gel using a Qiagen Gel Extraction kit, (2) digested with *EcoR* I and *Hind* III (New England BioLabs, Beverly, MA), and (3) ligated to pUC18 that had also been digested with *EcoR* I and *Hind* III and gel purified. The resulting construct designated appUC18-RSdds is depicted in Figure 19. The ligation was
25 carried out with T4 DNA ligase at 16°C for 16 hours. Once ligated, one µL of the ligation reaction was used to electroporate *E. coli* ElectroMAX™ DH10B™ cells (Life Technologies, Inc., Rockville, MD). The electroporated cells were plated onto LB-Amp plates (Amp concentration was 100 µg/mL). From these LB-Amp plates, eight individual colonies were selected at random, and the plasmids within these colonies were purified
30 using a Qiaprep Spin Miniprep kit. These purified plasmids were evaluated for the presence of inserts by restriction enzyme analysis. If the plasmids contained the correct

1.6 kb fragment, then an *EcoR* I and *Hind* III digest reaction would result in two fragments (2.6 and 1.6 kb), and a *Bam*HI digest reaction would result in one fragment (4.2 kb). After treating with the restriction enzymes, the digest reactions were separated by gel electrophoresis using a 0.8 percent TAE agarose gel. Of the eight clones tested,
 5 four contained the desired 1.6 kb fragment.

Example 4 – Cloning nucleic acid that encodes
 a *Sphingomonas trueperi* polypeptide having DDS activity

S. trueperi cells were grown as described in Example 1. In addition, genomic
 10 DNA was isolated from *S. trueperi* cells as described in Example 1.

To isolate nucleic acid encoding a polypeptide having DDS activity from *S. trueperi*, a strategy similar to that described in Example 3 was employed. In this case, four degenerate forward primers (SF1, SF2, SF3, and SF4) and four degenerate reverse primers (SR1, SR2, SR3, and SR4) were designed comparing sequences of several clones
 15 that encode polypeptides having polyprenyl diphosphate synthase activity (Figure 17). Codon usage tables from twelve *Sphingomonas* species were used to develop an average preferred codon table that was used in primer design. The sequence of each degenerate primer was as follows:

20 SF1: 5'-CTSSTSCAYGAYGAYGTSGTSGA-3' (SEQ ID NO:80)
 SF2: 5'-GTSGMVGSSGGSGGSAARC-3' (SEQ ID NO:81)
 SF3: 5'-CTSMTSCAYGAYGAYGTS-3' (SEQ ID NO:82)
 SF4: 5'-DSSRTBCTSGTSGGSGAYTT-3' (SEQ ID NO:83)
 SR1: 5'-VAKRAARTCCCSACSAGSAC-3' (SEQ ID NO:84)
 25 SR2: 5'-SACYTCSCCYTCGCRAT-3' (SEQ ID NO:85)
 SR3: 5'-RTCRTCSCCVAYVKTYTTSCC-3' (SEQ ID NO:86)
 SR4: 5'-SGGSAGSGTVRBYTTSCCYTC-3' (SEQ ID NO:87)

The primers were used in all logical combinations in PCR using Taq polymerase
 30 (Roche Molecular Biochemicals, Indianapolis, IN) and 1 ng of genomic DNA per microliter of reaction mix. PCR was conducted using the touchdown PCR program as

described in Example 1. Between about 4 μ M and 20 μ M of each PCR primer was used in each reaction depending on the degree of degeneracy. After each PCR reaction was complete, a portion of each reaction was separated by gel electrophoresis using a 1.5 percent TAE agarose gel. Each PCR reaction produced several amplified fragments of the expected sizes based on the coding sequences of other polyprenyl diphosphate synthase polypeptides. These fragments were isolated from TAE agarose gels and purified using the Qiagen Gel Extraction procedure (Qiagen Inc., Valencia, CA). An aliquot of each purified fragment was ligated into pCRII-TOPO. The ligated plasmids were then inserted into TOP10 *E. coli* cells using a TOPO cloning procedure (Invitrogen, Carlsbad, CA). The products of each of the individual insertion reactions were plated on LB-Amp-Xgal plates as described in Examples 1 and 3. Single white colonies that grew on the LB-Amp-Xgal plates were re-plated onto fresh LB-Amp-Xgal plates and screened in a PCR reaction using the initial degenerate primers to confirm the presence of the desired insert. Plasmid DNAs having the desired insert were obtained from multiple colonies using a QiaPrep Spin Miniprep Kit (Qiagen, Inc). The obtained plasmid DNAs were then quantified and sequenced using the M13 forward and reverse primers. Sequence analysis revealed that a 201 bp fragment produced using the SF1 and SR2 degenerate primers, a 476 bp fragment produced using the SF1 and SR4 primers, and a 206 bp fragment produced using the SF3 and SR2 primers contained sequences similar to the coding sequences of other polyprenyl diphosphate synthases.

Genome walking was performed to obtain a complete coding sequence for the *S. trueperi* DDS polypeptide using procedures similar to those described in Example 1. Briefly, primers were designed based on the sequences of the obtained fragments. These primers had the following sequences:

GSP5F: 5'-GTGCTGGTCGGCGACTTCCTGTTTCAG-3' (SEQ ID NO:88)
GSP6F: 5'-ATCGACCTGTCCGAGGATCGCTATCTC-3' (SEQ ID NO:89)
GSP5R: 5'-TCGAACGAGCGGCTGAACAGGAAGTC-3' (SEQ ID NO:90)
GSP6R: 5'-TGGCGGGATTGCCCCAGATGATGTTG-3' (SEQ ID NO:91)

The GSP5F and GSP6F primers are primers that face downstream of the DDS start codon, while the GSP5R and GSP6R primers are primers that face in the opposite direction. In addition, the GSP6F and GSP6R primers are nested inside the GSP5F and GSP5R primers.

5 Genome walking was conducted as described in Example 3 with the exception that the 36 cycles had 3 minute incubations at 66°C instead of 67°C and the final extension was performed at 66°C instead of 67°C for both the first and second rounds of PCR. Portions of the PCR reactions from each round were separated by gel electrophoresis using a 1.5 percent TAE agarose gel. PCR on the *Fsp* I and *Stu* I libraries
10 with the forward primers and of all four libraries with the reverse primers resulted in the production of an amplified fragment. A 1.4 kb fragment from the *Fsp* I library, a 1.1 kb fragment from the *Stu* I library (forward primer), a 2.0 kb fragment from the *Pvu* II library (forward primer), and a 3.0 kb fragment from the *Stu* I library (reverse primer) were gel purified, cloned using the TOPO cloning procedure, and sequenced as described
15 in Examples 1 and 3. The sequencing analysis revealed that these fragments contained sequences that overlapped with the sequence of the initially obtained fragments and were similar to the coding sequences of other polyprenyl diphosphate synthases. The full-length clone containing coding and non-coding sequence was 1833 bp in length (Figure 10). The open reading frame was 1014 bp in length (Figure 11), which encoded a
20 polypeptide with 337 amino acid residues (Figure 12).

The coding sequence of the DDS polypeptide from *S. trueperi* was amplified by PCR using *S. trueperi* genomic DNA as template. PCR primers were designed based on the sequences obtained as described above. The sequences of the primers were as follows.

25

SHDDSF: 5'-ATTAGGTACCATCAGATAATCGTCGCTCAA-3' (SEQ ID NO:92)
SHDDSR: 5'-TATAGGATCCGACATGGACGAGGAAGACGC-3' (SEQ ID NO:93)

30 These primers were designed to introduce a *Kpn* I restriction site at the beginning of the amplified fragment and a *Bam*H I restriction site at the end of amplified fragment. The sequence of each restriction site is underlined. The PCR reactions were performed as

described in Example 3 with the exception that primers SHDDSF and SHDDSR were used instead of RDS18F and RDS18R. Once the PCR was completed, the PCR reactions were separated by gel electrophoresis using a 0.8 percent TAE agarose gel. The gel electrophoresis revealed a 1.6 kb fragment. This 1.6 kb fragment was (1) purified using a
5 Qiagen Gel Extraction kit, (2) digested with *Kpn* I and *Bam*HI (New England BioLabs), and (3) ligated into pUC18 that had also been digested with *Kpn* I and *Bam*HI and gel purified using methods similar to those described in Example 3. The resulting construct designated appUC18-SHDDS is depicted in Figure 20. This construct was used to transform cells as described in Example 3. The transformed cells were plated onto LB-
10 Amp plates, and eight individual colonies were selected at random. Plasmid DNA was isolated from each colony using a QiaPrep Spin Miniprep kit. The extracted plasmid DNA was tested for the presence of the 1.6 kb fragment using three different restriction digests. If the plasmids contained the 1.6 kb fragment, then a *Bam*HI and *Kpn* I digest would yield two fragments (2.68 and 1.62 kb), an *Eco*R I digest would yield two
15 fragments (1.45 and 2.85 kb), and a *Ban* II digest would yield two fragments (0.48 and 3.8 kb). All eight plasmids tested yielded digestion fragments consistent with a plasmid containing the desired 1.6 kb fragment.

Example 5 – Measuring CoQ(10)

20 Harvested cells were suspended in water to have about 0.1 gm dry weight per mL. The suspension was subjected to a French-press, and the resulting in suspension was frozen in 1 mL aliquots until used.

To measure CoQ(10) in a sample, two aliquots were repeatedly thawed and refrozen 4-5 times. Once transferred to a 50 mL centrifuge tube, 1 mL of 5% sodium
25 dodecyl sulfate was added to the thawed material. The material was then flushed with nitrogen. After vortexing for one minute, six mL of ethanol was added to the material, and the resulting mixture was vortexed for one minute. Then, 15 mL of hexane was added to the mixture. After vortexing for five minutes, the mixture was centrifuged at
3000 rpm for ten minutes. Once centrifuged, the hexane layer was removed to a conical
30 flask and flushed with nitrogen. This hexane extraction was repeated two times. The three extracts were pooled into a single tube that was evaporated on a vacuum evaporator

until the residue was near dryness. The residue was dissolved in 2 mL of mobile phase by vortexing for 2-3 minutes. Once vortexed, the solution was transferred to a 5 mL volumetric flask. The tube that contained the residue was rinsed two additional times with 1 mL of mobile phase. Each time the rinse solution was transferred to the same 5 mL volumetric flask. After adjusting the total volume to 5 mL, the solution was mixed well and stored at -20°C until analyzed.

As a control, either water or a culture solution was spiked with standard CoQ(10), extracted as indicated above, and analyzed to determine the recovery of the spiked material. The CoQ(10) standard was a stock solution of CoQ(10), obtained from Sigma. The stock solution was made in HPLC grade ethanol at a concentration of 100 µg/mL, and then diluted to get CoQ(10) solutions ranging from 100 µg/mL to 1 µg/mL.

HPLC analysis was performed with the following parameters. The mobile phase was ethanol:methanol (7:3) or methanol:isopropylether (9:1). The flow rate was 0.75 mL/min. The column was Waters Nova-Pak C18 (3.9 x150 mm; 4Um). The detector was a PDA set from 200-300 nm with the resolution at 1.2 nm and the maximum absorbance at 275 nm. The run time was 15 minutes, and the injection volume was 50 µL. To calculate the amount of CoQ(10) present, 50 µL of each sample was injected, and the results compared to those obtained using the calibration curve. From these data points, the concentration per gm dry weight was calculated.

Example 6 – Introducing nucleic acid that encodes a polypeptide having DDS activity into cells and measuring isoprenoid levels

The following procedures were followed individually for the *R. sphaeroides* and *S. trueperi* nucleic acid isolated as described in Examples 3 and 4, respectively.

Plasmid DNA encoding the polypeptide having DDS activity was electroporated into wild type *E. coli* strain MG1655. The electroporated cells were plated onto LB-Amp plates. A single individual bacterial colony was picked for each DDS coding sequence, and each colony was grown overnight in 2 mL of LB-Amp at 37°C with 200 rpm shaking. About 0.75 mL of these overnight cultures were used to inoculate flasks containing 75 mL LB-Amp medium (Amp concentration was 100 µg/mL). These second cultures were grown at 37°C at 200 rpm for 30 hours. Additional Amp (to a final concentration of 50

µg of fresh Amp per mL) was added to each flask after 12 hours of growth. After 30 hours, the bacteria were collected by centrifugation at 8,000 g for 10 minutes. The resulting bacterial cell pellets were washed by adding 20 mL of 10 mM Tris-HCL buffer (pH 8.0), resuspending the cells, and re-centrifuging as before. Each cell pellet was then
5 resuspended in 10 mL of water. About 0.5 mL of each extract was used for dry mass analysis and the remaining cell suspensions (about 9.5 mL) were frozen at -20°C overnight.

The 9.5 mL cell suspensions were used as follows. First, the cells were thawed on ice and lysed by passing the cell suspensions through a French press three times (14,000
10 psi pressure). The resulting cell extracts were frozen at -20°C in 1 mL aliquots and maintained on ice prior to analysis.

High pressure liquid chromatography was performed using Waters' 2690 Alliance integrated system (Waters Corporation, Milford, Mass). Prior to analysis, all samples and standards were dissolved in HPLC-grade ethanol, loaded into the built-in auto-sampler,
15 and kept at 5°-10°C in the dark. The separation was carried out using an isocratic elution program of 70:30 ethanol/methanol (v/v) at a flow rate of 1.0 mL/min. The column was a Waters Nova-Pak C18, 3.9-150 mm equipped with a guard column of the same stationary phase. The injection volume was typically 10-25 µL. Total run time was ten minutes.

Under these conditions, retention times were 3.1 and 4.9 minutes for CoQ(8) and
20 CoQ(10), respectively. For quantification purposes, a four-point external calibration curve was calculated using freshly prepared CoQ(10) standards. Calibration levels were 1.0, 4.0, 10.0 and 100.0 µg/mL (ppm). Each standard was injected in triplicate, and the resulting calibration plot was linearly fitted with observed r^2 's of >0.999.

For UV and MS detection, a photodiode array (PDA, Model UV6000LP,
25 ThermoQuest Corp., San Jose, CA) and an ion trap mass analyzer (LCQ Classic, Finnigan/ThermoQuest Corp., San Jose, CA) were connected in series with the chromatograph and without splitting of the effluent. The PDA was operated in scanning mode from 220-300 nm. Effluent from the PDA was introduced into the mass analyzer via atmospheric-pressure chemical ionization (APCI) using the following parameters:
30 capillary temperature, 150°C; capillary voltage, 3kV; vaporizer temperature, 400°C; sheath gas (N₂) flow, 80 arbitrary units; auxiliary gas (N₂) flow, 5 arbitrary units; and

corona discharge needle, 5mA/6kV. Positive-ion detection was performed in full scan (250-1000 m/z), 2 mscans, 500 ms ion injection time.

Under these conditions, CoQ(8) yielded a mass spectrum with a base peak at 727.5 m/z, corresponding to the protonated 'molecular ion' as well as several satellite ions from ethanol and/or methanol adducts (Figures 23 and 24). Similarly, CoQ(10) yielded a mass spectrum with a base peak at 863.6 m/z corresponding to its protonated 'molecular ion' (Figure 25). Several ethanol and/or methanol satellite adducts were observed as well. Both CoQ(8) and CoQ(10) yielded UV spectra with maxima at 274 nm.

Two samples were analyzed: MG1655 PUC18 and MG1655 PUC18-DDS. MG1655 PUC18 is *E. coli* strain MG1655 transfected with the PUC18 vector only. MG1655 PUC18-DDS is *E. coli* strain MG1655 transfected with the PUC18 vector containing nucleic acid that encodes a *R. sphaeroides* polypeptide having DDS activity. The MG1655 PUC18 specimen contained only CoQ(8) (retention time 3.08 min, Figure 21) as confirmed by its mass spectrum (Figure 23), with a base peak at 727.4 m/z and a UV spectrum with a maximum at 274 nm. The MG1655 PUC18-DDS specimen, however, contained CoQ(8) and CoQ(10) (Figure 22), both of which were confirmed by matching mass spectra (Figures 24 and 25) and UV maxima.

Example 7 – Cloning nucleic acid that encodes
a *Sphingomonas trueperi* polypeptide having DXR activity

Sphingomonas trueperi ATCC 12417 cultures (100-200 mL) were grown in nutrient broth at 30°C and 250 rpm for 2-3 days. The cells then were pelleted and washed with a 10 mM Tris:1.0 mM EDTA solution. The pellets were resuspended in 5 mL of GTE buffer (50 mM glucose, 25 mM Tris HCl (pH 8.0), 10 mM EDTA (pH 8.0)) per 100 mL of culture. Lysozyme and Proteinase K were added to a 1 mg/mL concentration and mutanolysin was added to 5.5 µg/mL. After a 1.5 hour incubation at 37°C, SDS was added to a final concentration of 1%, and the concentration of Proteinase K was brought to 2 mg/mL. After incubation at 50°C for one hour, an equal volume of GTE buffer was added, and NaCl was added to a 0.15 M concentration. The mixture was extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and centrifuged at

10,000 rpm for 10 minutes. The supernatant was removed to a clean tube, extracted with an equal volume of chloroform, and centrifuged at 5,000 rpm for 10 minutes. The supernatant was treated with RNase and precipitated with 2.5 volumes of ethanol. The spooled DNA was washed with 70% ethanol, air dried, and resuspended in 10 mM Tris (pH 8.5). After resuspending, the resuspended DNA was further cleaned by re-extraction with phenol:chloroform:isoamyl alcohol and chloroform, and reprecipitation with 1/10 volume 7.4 M NH₄OAc and 2.5 volumes ethanol.

A conserved region of the 1-deoxy-D-xylulose 5-phosphate reductoisomerase (dxr) gene was cloned by PCR. Five degenerate forward and five degenerate reverse PCR primers were designed from conserved protein regions that were revealed by aligning known dxr genes (Figure 27). The degenerate sequences were designed from the conserved regions using the universal codon table. The primers were used in all logical combinations in PCR using Taq polymerase (Roche Molecular Biochemicals, Indianapolis, IN) and 1 ng of genomic DNA/ μ L reaction mix. PCR was conducted using a touchdown PCR program with 4 cycles at an annealing temperature of 59°C, 4 cycles at 57°C, 4 cycles at 55°C, and 24 cycles at 53°C. Each cycle used an initial 30 second denaturing step at 94°C and a 1.75 minute extension at 72°C, and the program had an initial denaturing step for 2 minutes at 94°C and final extension of 5 minutes at 72°C. The amounts of PCR primer used in the reaction were increased 3-12 fold above typical PCR amounts depending on the amount of degeneracy in the 3' end of the primer. In addition, separate PCR reactions containing each individual primer were made to identify PCR products resulting from single degenerate primers. Fifteen μ L of each PCR product was separated on a 1.5% TAE (Tris-acetate-EDTA)-agarose gel. Degenerate primers F2 (5'-CCSGTSGAYWSSGARCAYAACGCS-3' (SEQ ID NO:132)) and R7 (5'-ATGATGAACAAGGGSCTSGAR-3' (SEQ ID NO:133)) produced a band of about 250 bp, which was the expected size based on dxr genes from other species. This band was not present in the individual F2 and R7 primer control reactions. Degenerate primers F3 (5'-CATCCVAACTGGWMVATGGG-3' (SEQ ID NO:134)) and R2 (5'-ATYGGYRWWCKCATATCMGG-3' (SEQ ID NO:135)) produced a band of about 200 bp, which also was the expected size. The F2-R7 and F3-R2 fragments were isolated and purified using a QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, CA). Three μ L of

the purified band was ligated into pCR[®]II-TOPO vector, which was then transformed by a heat-shock method into TOP10 *E. coli* cells using a TOPO cloning procedure (Invitrogen, Carlsbad, CA). Transformations were plated on LB media containing 100 µg/mL of ampicillin and 50 µg/mL of 5-Bromo-4-Chloro-3-Indolyl-B-D-Galactopyranoside (X-gal). Individual, white colonies were resuspended in about 20 µL of 10 mM Tris and heated for 10 minutes at 95°C to break open the bacterial cells. To screen individual colonies, 2 µL of the heated cells was used in a 25 µL PCR reaction as described above using the appropriate degenerate primers. Plasmid DNA was obtained with a QIAprep Spin Miniprep Kit (Qiagen, Inc) from cultures of colonies having the desired insert and used for DNA sequencing with M13R and M13F primers. Sequence analysis revealed that the F2-R7 and F3-R2 fragments overlapped and were homologous to known *dxr* genes.

Genome walking was performed to obtain the complete coding sequence as follows. The overlapping of the F2-R7 and F3-R2 fragments resulted in a sequence 358 bp in length. The following four primers for conducting genome walking in both upstream and downstream directions were designed using the portion of this sequence that was internal to the degenerate primers:

GSP1F 5'-CGAATGGACGACGGATTGGCGATGGAC-3' (SEQ ID NO:136)
 GSP2F 5'-TCAGTTCGAGCCCCTTGTTTCATCATCGTC-3' (SEQ ID NO:137)
 GSP1R 5'-CGAACTGATCGAAGCCTTCCACCTGTTC-3' (SEQ ID NO:138)
 GSP2R 5'-GGTCCATCGCCAATCCGTCGTCCATTC-3' (SEQ ID NO:139)

The GSP1F and GSP2F primers faced upstream, the GSP1R and GSP2R primers faced downstream, and the GSP2F and GSP2R primers were nested inside the GSP1F and GSP1R primers. Genome walking was conducted according to the manual for CLONTECH's Universal Genome Walking Kit (CLONTECH Laboratories, Inc., Palo Alto, CA) with the exception that the enzymes *FspI* and *SmaI* were used in place of *DraI* and *EcoRV*. The *DraI* and *EcoRV* enzymes were replaced because they cut *S. trueperi* genomic DNA too infrequently to give fragment lengths amenable to PCR. The PCR mixture contained 5% DMSO. First round PCR was conducted in a Perkin Elmer 9700

Thermocycler with 7 cycles consisting of 2 seconds at 94°C and 3 minutes at 72°C, and 36 cycles consisting of 2 seconds at 94°C and 3 minutes at 66°C, with a final extension at 66°C for 4 minutes. Second round PCR used 5 cycles consisting of 2 seconds at 94°C and 3 minutes at 72°C, and 26 cycles consisting of 2 seconds at 94°C and 3 minutes at 66°C, with a final extension at 66°C for 4 minutes. Nine µL of the first round product and seven µL of the second round product were separated on a 1.5% TAE-agarose gel. A 1.3 Kb band was obtained from the second round product for the SmaI forward reaction, an 800 bp band for the StuI reverse reaction, and a 750 bp band for the PvuII reverse reaction. These fragments were gel purified, cloned, and sequenced. Internal primers were used to amplify and obtain additional sequence of the gene. Sequence analysis revealed that the sequence derived from genome walking overlapped with the original fragments and contained an entire coding sequence homologous to known dxr genes. The full-length clone containing coding and non-coding sequence was 2017 bp in length (Figure 28). The open reading frame starting with the first GTG site was 1161 bp in length (Figure 29), which encoded a polypeptide with 386 amino acid residues (Figure 30).

Example 8 – Making recombinant microorganisms

Rhodobacter sphaeroides (ATCC 35053) was routinely maintained on Luria
Bretain (Miller) agar (Fisher scientific) plates. When needed, *R. sphaeroides* was
cultured as follows. A 5 mL culture was grown in a 15 mL culture tube at 30°C in Innova
4230 Incubator, Shaker (New Brunswick Scientific, Edison, NJ) with a shaking speed of
250 rpm. Each 5 mL culture was started by inoculating liquid media (Sistrom media
supplemented with 20% LB) with a single colony. The liquid media contained the
following ingredients per liter: 2.72 g KH₂PO₄, 0.5 g (NH₄)₂SO₄, 0.5 g NaCl, 0.2 g EDTA
disodium salt, 0.3 g MgSO₄ · 7H₂O, 0.033 g CaCl₂ · 2H₂O, 0.2 mg FeSO₄ · 7H₂O, 0.02
mL (NH₄)₆Mo₇O₂₄ · 4H₂O (1% solution), 1 mL Trace element solution, 0.2 mL Vitamin
solution, 5 g Luria Bretain Broth Mix, and 8 mL Glucose (50%). The Trace element
solution contained the following ingredients per liter: 1.765 g EDTA disodium salt, 10.95
g ZnSO₄ · 7H₂O, 5 g FeSO₄ · 7H₂O, 1.54 g MnSO₄ · H₂O, 0.392 g CuSO₄ · 5H₂O, 0.284 g
Co(NO₃)₂ · 6H₂O, and 0.114 g H₃BO₃. The Vitamin solution contained the following

ingredients per liter: 10 g Nicotinic acid, 5 g Thiamine HCl, and 0.01 g Biotin. The vitamins and glucose were added after the media cooled to room temperature after autoclaving. When necessary, the media was supplemented with one or more of the following antibiotics: Kanamycin (25 µg/mL; final concentration), Spectinomycin (25 µg/mL; final concentration), and/or Streptomycin (25 µg/mL; final concentration).

Electrocompetent *R. sphaeroides* cells

Electrocompetent *R. sphaeroides* cells were made as follows. A 5 mL culture of *R. sphaeroides* was grown overnight at 30°C in Sistrom's media supplemented with 20% LB. This culture was diluted 1/100 in 300 mL of the same media and grown to an OD₆₆₀ of 0.5-0.8. The cells were chilled on ice for 10 minutes and then centrifuged for 6 minutes at 7,500 g. The supernatant was discarded, and the cell pellet was resuspended in ice-cold 10% glycerol at half of the original volume. The cells were pelleted by centrifugation for 6 minutes at 7,500 g. The supernatant was again discarded, and cells resuspended in ice-cold 10% glycerol at one quarter of the original volume. The last centrifugation and resuspension steps were repeated, followed by centrifugation for 6 minutes at 7,500 g. The supernatant was decanted, and the cells resuspended in the small volume of glycerol that did not drain out. Additional ice-cold 10% glycerol was added to resuspend the cells, if necessary. Forty µL of the resuspended cells was used in a test electroporation to determine if the cells needed to be concentrated by centrifugation or diluted with 10% ice-cold glycerol. Time constants of 8.5-9.0 milliseconds resulted in good transformation efficiencies. If cells were too dilute, the time constant was greater than 9.0 and transformation efficiencies were low. If cells were too concentrated, the electroporation would spark. Once an acceptable time constant was achieved, cells were aliquoted into cold microfuge tubes and stored at -80°C. All water used for media and glycerol was 18.2 Mohm-cm or higher.

Electrocompetent *R. sphaeroides* cells were electroporated as follows. One µL of plasmid DNA was gently mixed into 40 µL of *R. sphaeroides* electrocompetent cells, which were then transferred to an electroporation cuvette with a 0.2 cm electrode gap. Electroporations were conducted using a Biorad Gene Pulser II (Biorad, Hercules, CA) with settings at 2.5 kV of energy, 400 ohms of resistance, and 25 µF of capacitance. Cells

were recovered in 400 μ L SOC media at 30°C for 6-16 hours. The cells were then plated (200 μ L per plate) on the appropriate selective media. Transformation efficiencies averaged about 2,000 transformants/ μ g of DNA.

5 Electrocompetent *E. coli* cells

Electrocompetent *E. coli* strain S17-1 cells were made as follows. A 5 mL culture of *E. coli* strain S17-1 was grown overnight at 30°C in LB media supplemented with 25 μ g/mL of streptomycin and 25 μ g/mL of spectinomycin. This culture was diluted 1/100 in 300 mL of the same media and grown to an OD₆₆₀ of 0.5-0.8. The cells were chilled on
10 ice for 10 minutes and then centrifuged for 6 minutes at 7,500 g. The supernatant was discarded, and the cell pellet was resuspended in ice-cold 10% glycerol at half of the original volume. The cells were pelleted by centrifugation for 6 minutes at 7,500 g. The supernatant was again discarded, and the cells were resuspended in ice-cold 10% glycerol at one quarter of the original volume. The last centrifugation and resuspension steps were
15 repeated, followed by centrifugation for 6 minutes at 7,500 g. The supernatant was decanted, and the cells resuspended in the small volume of glycerol that did not drain out. Additional ice-cold 10% glycerol was added to resuspend the cells, if necessary. Cells were aliquoted into cold microfuge tubes and stored at -80°C.

Electrocompetent *E. coli* strain S17-1 cells were electroporated as follows. Forty
20 μ L of competent cells was used per electroporation. Electroporation was conducted using a Biorad Gene Pulser II and a standard *E. coli* protocol: 2.5 kV of energy, 200 ohms of resistance, and 25 μ F of capacitance. Electroporated cells were recovered in 250-1000 μ L of SOC media for one hour, and 10-200 μ L of culture was plated per plate of selective media. Transformation efficiencies averaged about 1.5×10^4 transformants/ μ g of DNA.

25

Constructs

Various clones were overexpressed in *R. sphaeroides* using the broad-host-range vector pBBR1MCS2 (Kovach *et al.*, *Gene*, 166:175-176 (1995)) that was engineered to have either an *R. sphaeroides* *rrnB* promoter, an *R. sphaeroides* *glnB* promoter, or a tet
30 promoter. The pBBR1MCS2 vector is mobilizable and relatively small (5,144 bp), replicates in *R. sphaeroides*, has a multiple cloning site with lacZ α color selection, and

carries a kanamycin resistance gene. All restriction enzymes and T4 DNA ligase were obtained from New England Biolabs (Beverly, MA) unless otherwise indicated. All plasmid DNA preparations were done using QIAprep Spin Miniprep Kits or Qiagen Maxi Prep Kits, and all gel purifications were done using QIAquick Gel Extraction Kits (Qiagen, Valencia, CA).

pMCS2rrnBP

The vector designated pMCS2rrnBP, which contains an *R. sphaeroides* rrnB promoter, was constructed by inserting a copy of the *R. sphaeroides* rrnB promoter (rrnBP) into the pBBR1MCS2 vector. The rrnB promoter was isolated from the pTEX124 vector (obtained from S. Kaplan) by digestion with the restriction enzyme BamHI, which releases the promoter as a 363 bp fragment. Alternatively, the rrnB promoter can be obtained by PCR amplifying it from *R. sphaeroides* genomic DNA using primers based on published rrnB sequence (GenBank® accession number X53854). This fragment was gel purified from a 2% Tris-acetate-EDTA (TAE) agarose gel. The pBBR1MCS2 vector was also digested with BamHI, and the enzyme heat inactivated at 80°C for 20 minutes. The digested vector was then dephosphorylated with shrimp alkaline phosphatase (Roche Molecular Biochemicals, Indianapolis, IN) and gel purified from a 1% TAE-agarose gel. The prepared vector and the rrnBP fragment were ligated using T4 DNA ligase at 16°C for 16 hours. One µL of ligation reaction was used to electroporate 40 µL of *E. coli* Electromax™ DH10B™ cells (Life Technologies, Inc., Rockville, MD). Electroporated cells were plated on LB media containing 25 µg/mL of kanamycin (LBK). Plasmid DNA was isolated from cultures of single colonies and was digested with HindIII restriction enzyme to confirm the presence of a single insertion of the rrnB promoter. The sequence of the rrnBP inserts for these colonies was also confirmed by DNA sequencing.

pMCS2glnBP

The vector designated pMCS2glnBP, which contains an *R. sphaeroides* glnB promoter, was constructed by inserting a copy of the *R. sphaeroides* glnB promoter (glnBP) into the pBBR1MCS2 vector. The glnB promoter was PCR amplified from

genomic DNA obtained from *R. sphaeroides* strain 35053. The following primers were designed based on sequence information obtained from GenBank® accession number X71659:

- 5 glnBF 5'-ATTATCTAGAATCCGCCCCGCCTCCACCTC-3' (SEQ ID NO:140)
 glnBR 5'-GATGGATCCTGGGTAGGGTCGCTGCTGTCC-3' (SEQ ID NO:141)

The primers introduced an XbaI restriction site at the 5' end and a BamHI restriction site at the 3' end. The following reaction mix and PCR program was used to
 10 amplify the promoter region of the glnB gene.

Reaction Mix		PCR program
15	Pfu 10X buffer	10 µL
	DMSO	5 µL
	dNTP mix (10 mM)	4 µL
	glnBF (50 µM)	2 µL
	glnBP (50 µM)	2 µL
	Genomic DNA (50ng/µL)	2 µL
	Pfu enzyme (2.5 U/µL)	2 µL
	DI water	73 µL
	Total:	100 µL
		94°C 2 minutes
20		7 cycles of:
		94°C 30 seconds
		61°C 45 seconds
		72°C 3 minutes
		25 cycles of:
		94°C 30 seconds
		66°C 45 seconds
		72°C 3 minutes
		72°C 7 minutes
		4°C Until used further

- 25 The PCR product was separated on a 1.2% TAE-agarose gel. An about 500 bp fragment was excised and gel purified. The isolated DNA was restricted with XbaI and BamHI, and the resulting digested DNA column purified using a Qiagen gel isolation kit. Three µg of pBBR1MCS2 plasmid DNA was digested with BamHI and XbaI. The digestion was inactivated at 80°C for 20 minutes. The digested vector was then
 30 dephosphorylated with shrimp alkaline phosphatase and gel purified on a 1% TAE-agarose gel. Eighty-six ng of the prepared pBBR1MCS2 vector was ligated with 60 ng of the digested glnBP PCR product using T4 DNA ligase at 14°C for 14-16 hours. One µL of ligation reaction was used to electroporate 40 µL of *E. coli* Electromax™ DH10B™ cells. Electroporated cells were plated on LB media containing 25 µg/mL of kanamycin
 35 and 50 µg/mL of Xgal (LBKX). Eight individual, white colonies were selected, and their

plasmid DNA isolated using a QIAprep Spin Miniprep Kit. Plasmid DNA isolated from each colony was digested in separation reaction mixtures with PstI and a combination of EcoRI/XbaI. All eight clones had a restriction pattern that indicated the presence of the insert. The sequence of three clones was verified.

5

pMCS2tetP

The vector designated pMCS2tetP, which contains a tet promoter, was constructed by cloning the promoter for the tetracycline resistance determinants from transposon Tn1721 (Waters *et al.*, *Nucleic Acids Research*, 11(17):6089-6105 (1983)) into the
 10 pBBR1MCS2 vector. The tetA gene promoter (tetP) was amplified using plasmid pRK415 as template. The following primers were designed to introduce an XbaI restriction site at the beginning of the amplified fragment and a BamHI site at the end of the amplified fragment.

15

TETXBAF 5'-TTATCTAGAACCGTCTACGCCGACCTC-
 GTTCAAC-3' (SEQ ID NO:142)

TETBAMR 5'-TTAGGATCCCCTCCGCTGGTCCGATTG-
 AAC-3' (SEQ ID NO:143)

20

The PCR mix contained the following: 1X Native Plus Pfu buffer, 20 ng pRK415 plasmid DNA, 0.2 μ M of each primer, 0.2 mM of each dNTP, 5% DMSO (v/v), and 10 units of native Pfu DNA polymerase in a final volume of 200 μ L. The PCR reaction was performed in a Perkin Elmer Geneamp PCR System 2400 under the following conditions: an initial denaturation at 94°C for 1 minute; 8 cycles of 94°C for 30 seconds, 60°C for 45
 25 seconds, and 72°C for 45 seconds; 24 cycles of 94°C for 30 seconds, 66°C for 45 seconds, and 72°C for 45 seconds; and a final extension for 7 minutes at 72°C. The amplification product was then separated by gel electrophoresis using a 2 %TAE-agarose gel. A 160 bp fragment was excised from the gel and purified. The purified fragment was digested simultaneously with XbaI and BamHI restriction enzymes, and purified with
 30 a QIAquick PCR Purification Kit. Three μ g of pBBR1MCS2 plasmid DNA was digested with BamHI and XbaI, and the digest was inactivated at 80°C for 20 minutes. The

digested vector was then dephosphorylated with shrimp alkaline phosphatase and gel purified on a 1% TAE-agarose gel.

100 ng of the prepared pBBR1MCS2 vector was ligated with 36 ng of the digested tetP PCR product using T4 DNA ligase at 16°C for 16 hours. One µL of ligation reaction
5 was used to electroporate 40 µL of *E. coli* Electromax™ DH5α™ cells. Electroporated cells were plated on LB media containing 25 µg/mL of kanamycin and 50 µg/mL of Xgal (LBKX). Individual, white colonies were resuspended in about 25 µL of 10 mM Tris, and 2 µL of the resuspension was plated on LBKX. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells. Two µL of the heated
10 cells was used in a 25 µL PCR reaction using the following primers homologous to the vector and flanking the cloning site:

MCS2FS 5'-AGGCGATTAAGTTGGGTAAC-3' (SEQ ID NO:144)

MCS2RS 5'-GACCATGATTACGCCAAG-3' (SEQ ID NO:145)

15

The PCR mix contained the following: 1X Taq PCR buffer, 0.2 µM each primer, 0.2 mM each dNTP, and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 32 cycles of 94°C for 30 seconds, 55°C for 45
20 seconds, and 72°C for 1 minute; and a final extension for 7 minutes at 72°C. All colonies showed a single insertion event. Plasmid DNA was isolated from cultures of two individual colonies and sequenced to confirm the DNA sequence of the tet promoter in the construct.

25 pMCS2rrnBP/Stdxs

The nucleic acid encoding a *S. trueperi* polypeptide having DXS activity was cloned in the pMCS2rrnBP vector as follows. The *S. trueperi* dxs gene was amplified by PCR using primers homologous to sequence upstream and downstream of the gene. These primers, STDXSMCSF and STDXSMCSR, were designed to introduce a ClaI
30 restriction site at the beginning of the amplified fragment and a KpnI site at the end of the amplified fragment.

STDXSMCSF 5'-GATAATCGATGTGTGACTGACCTGT-
CCAAC-3' (SEQ ID NO:146)

STDXSMCSR 5'-CTTAGGTACCATGTTGGAGATTCAA-
GGTGG-3' (SEQ ID NO:147)

5

The PCR mix contained the following: 1X Native Plus Pfu buffer, 200 ng *S. trueperi* genomic DNA, 0.2 µM of each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 10 units of native Pfu DNA polymerase (Stratagene, La Jolla, CA) in a final volume of 200 µL. The PCR reaction was performed in a Perkin Elmer Geneamp PCR System 10 2400 under the following conditions: an initial denaturation at 94°C for 1 minute; 8 cycles of 94°C for 30 seconds, 54°C for 45 seconds, and 72°C for 3.5 minutes; 27 cycles of 94°C for 30 seconds, 60°C for 45 seconds, and 72°C for 3.5 minutes; and a final extension for 7 minutes at 72°C. The amplification product was then separated by gel electrophoresis using a 1% TAE-agarose gel. A 2.2 Kb fragment was excised from the 15 gel and purified. The purified fragment was digested with ClaI restriction enzyme, purified with a QIAquick PCR Purification Kit, digested with KpnI restriction enzyme, purified again with a QIAquick PCR Purification Kit, and quantified on a minigel.

Three µg of the pMCS2rrnBP vector was digested with the restriction enzyme ClaI, gel purified on a 1% TAE-agarose gel, digested with KpnI, purified with a 20 QIAquick PCR Purification Kit, dephosphorylated with shrimp alkaline phosphatase, and purified again with a QIAquick PCR Purification Kit. 120 ng of the digested PCR product containing the *S. trueperi* dxs gene and the 50 ng of the prepared pMCS2rrnBP vector was ligated using T4 DNA ligase at 16°C for 16 hours. One µL of the ligation reaction was used to electroporate 40 µL of *E. coli* Electromax™ DH10B™ cells. The 25 electroporated cells were plated onto media. Plasmid DNA was isolated from cultures of individual colonies and evaluated for the presence of the desired insert by restriction enzyme analysis with HindIII and SacI enzymes. The sequence of the Stdxs insert was confirmed by DNA sequencing. The resulting plasmid containing the Stdxs sequence under the control of the rrnB promotor was designated pMCS2rrnBP/Stdxs.

30 Purified pMCS2rrnBP/Stdxs plasmid DNA derived from a colony having the correct sequence was then electroporated into electrocompetent cells of *R. sphaeroides*

strain 35053. Plasmid DNA was isolated from cultures of individual *R. sphaeroides* colonies. Restriction patterns of plasmid preparations from *R. sphaeroides* are difficult to analyze due to the presence of multiple native plasmids in this species. To check the plasmid integrity in *R. sphaeroides*, one μ L of the plasmid preparation from a transformed
 5 *R. sphaeroides* colony was used to re-transform *E. coli* Electromax™ DH10B™ cells by electroporation. Electroporated cells were plated on LBK media. Plasmid DNA was isolated from cultures of individual colonies and evaluated using SacI and HindIII restriction digests.

10 pMCS2rrnBP/Stdxs2

A second pMCS2rrnBP plasmid containing the nucleic acid encoding a *S. trueperi* polypeptide having DXS activity was constructed. This construct was made using the following forward primer designed to introduce the ribosomal binding site (rbs) from the *R. sphaeroides* dxs1 gene along with a ClaI restriction site.

15

SXSCLAF2 5'-ACTATCGATGAAGGAAGAGCATGGCTGACCT-
 ACCCAAGAC-3' (SEQ ID NO:146)

S. trueperi genomic DNA was used as template in a PCR mixture using the
 20 primers SXSCLAF2 and STDXSMCSR. The PCR program and reaction mixture used were identical to those described for the pMCS2rrnBP/Stdxs construct. The PCR product was gel purified, digested with ClaI, purified with a QIAquick PCR Purification Kit, digested with restriction enzyme KpnI, and purified again with a QIAquick PCR
 Purification Kit. 150 ng of digested PCR product was ligated into 50 ng of the prepared
 25 pMCS2rrnBP vector using T4 DNA ligase at 16°C for 16 hours. One μ L of the ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells, and the electroporated cells were plated onto LBK plates. Plasmid DNA was isolated from cultures of individual colonies and evaluated for the presence of the desired insert by restriction enzyme analysis with HindIII and SacI enzymes. The sequence of the dxs
 30 insert was confirmed by DNA sequencing. The resulting plasmid containing the Stdxs

sequence under the control of the *rrnB* promotor and having an *R. sphaeroides* ribosomal binding site was designated pMCS2rrnBP/Stdxs2.

A confirmed construct was electroporated into *R. sphaeroides* strain 35053, and the electroporated cells were plated onto LBK media. Individual colonies were resuspended in about 25 μ L of 10 mM Tris, and 2 μ L of the resuspension was plated on LBK media. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and two μ L of the heated cells used in a 25 μ L PCR reaction using the SXSClaf2 and STDxSMCSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μ M each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase (Roche) per reaction. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 54°C for 1 minute, and 72°C for 3.5 minutes; 24 cycles of 94°C for 30 seconds, 60°C 1 minute, and 72°C for 3.5 minutes; and a final extension for 7 minutes at 72°C.

pMCS2rrnBP/Rsdds

The nucleic acid encoding a *R. sphaeroides* polypeptide having DDS activity was cloned in the pMCS2rrnBP vector as follows. The *R. sphaeroides* dds gene was PCR amplified using the following primer pair:

RDS18F 5'-ACTAGAATTCCGCAACAGTTCCTTCATGTC-3' (SEQ ID NO:147)
RSDDSMCSR 5'-CTAGATCGATACTTGCGGTCGGACTGATAG-3' (SEQ ID NO:148)

The forward primer was located upstream of the start codon and introduced an EcoRI restriction site, while the reverse primer was located downstream of the stop codon and introduced a ClaI restriction site. Since the forward primer was located upstream, the *R. sphaeroides* dds maintained its native ribosomal binding site. The following reaction mix and PCR program were used to amplify the *R. sphaeroides* dds gene.

Reaction Mix		Program
5	Pfu 10X buffer	10 μ L
	DMSO	5 μ L
	dNTP mix (10 mM)	4 μ L
	RDS18F (50 μ M)	2 μ L
	RSDDSMCSR (50 μ M)	2 μ L
10	Genomic DNA (50 ng/ μ L)	2 μ L
	Pfu enzyme (2.5 U/ μ L)	1 μ L
	DI water	74 μ L
Total:		100 μ L
		94°C 2 minutes
		8 cycles of:
		94°C 30 seconds
		55°C 45 seconds
		72°C 3 minutes
		21 cycles of:
		94°C 30 seconds
		61°C 45 seconds
		72°C 3 minutes
		72°C 7 minutes
		4°C Until used further

The PCR product was separated on a 1% TAE-agarose gel, and an about 1.8 Kb fragment was excised and gel purified. The isolated DNA was restricted with EcoRI and ClaI, and was column purified using a Qiagen gel isolation kit. Three μ g of pMCS2rrnBP vector DNA was digested with EcoRI, and the linear DNA was gel isolated using a Qiagen gel isolation kit. The vector was further digested with ClaI, and the DNA was column purified. The double-digested vector was then dephosphorylated with shrimp alkaline phosphatase and purified using a QIAquick PCR Purification Kit. The EcoRI/ClaI-digested *R. sphaeroides* dds PCR product was ligated into the prepared vector using T4 DNA ligase for 14-16 hours at 16°C. One μ L of the ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK (25 μ g/mL) media. Individual colonies were resuspended in about 25 μ L of DI water, and 2 μ L of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μ L of the heated cells was used in a 25 μ L PCR reaction using the RDS18F and RSDDSMCSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μ M each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed under the following conditions: an initial denaturation at 94°C for 2 minutes; 6 cycles of 94°C for 30 seconds, 55°C for 45 seconds, and 72°C for 3 minutes; 25 cycles of 94°C for 30 seconds, 61°C 45 seconds, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. The resulting plasmid containing the Rsdds sequence under the control of the rrnB promoter was designated pMCS2rrnBP/Rsdds.

The pMCS2rrnBP/Rsdds plasmid was electroporated into *E. coli* strain S17-1. This strain contains a chromosomal copy of the trans-acting elements that mobilize oriT-containing plasmids during conjugation with a second bacterial strain. It also carries a gene conferring resistance to the antibiotics streptomycin and spectinomycin.

- 5 Using the S17-1 strain, the pMCS2rrnBP/Rsdds plasmid was transferred to *R. sphaeroides* 35053 by conjugation. Individual colonies were purified by restreaking on LBK plates. Single colonies were screened by PCR using the RDS18F and RSDDSMCSR primers to confirm the presence of the insert as described above.

10 pMCS2rrnBP/Stdds

The nucleic acid encoding a *S. trueperi* polypeptide having DDS activity was cloned in the pMCS2rrnBP vector as follows. The *S. trueperi* dds gene was PCR amplified using the following primer pair:

- 15 STDDSMCSF 5'-GTCGCTCGAGATCAGATAATCGTCGCTCAA-3' (SEQ ID NO:149)
STDDSMCSR 5'-ATATGGTACCGACATGGACGAGGAAGACGC-3' (SEQ ID NO:150)

- 20 The forward primer was located upstream of the start codon and introduced a XhoI restriction site, while the reverse primer was located downstream of the stop codon and introduced a KpnI restriction site. Since the forward primer was located upstream, the *S. trueperi* dds fragment maintained its native ribosomal binding site. The following reaction mix and PCR program were used to amplify the *S. trueperi* dds gene.

25

<u>Reaction Mix</u>		<u>Program</u>	
Pfu 10X buffer	10 μ L	94°C	2 minutes
DMSO	5 μ L	8 cycles of:	
dNTP mix (10 mM)	4 μ L	94°C	30 seconds
30 SHDDSMCSF (50 μ M)	2 μ L	55°C	45 seconds
SHDDSMCSR (50 μ M)	2 μ L	72°C	3 minutes
Genomic DNA (50 ng/ μ L)	2 μ L	21 cycles of:	
Pfu enzyme (2.5 U/ μ L)	1 μ L	94°C	30 seconds
DI water	74 μ L	61°C	45 seconds

Total:	100 μ L	72°C 3 minutes
		72°C 7 minutes
		4°C Until used further

5 The PCR product was separated on a 1% TAE-agarose gel, and an about 1.6 Kb fragment was excised. The DNA was isolated using a Qiagen gel isolation kit. The isolated DNA was restricted with XhoI and KpnI, and was column purified using a Qiagen gel isolation kit. Two μ g of pMCS2rrnBP vector DNA was digested with KpnI, and the linear DNA was gel isolated using a Qiagen gel isolation kit. The vector was

10 further digested with XhoI, and the DNA was column purified. The double-digested vector was then dephosphorylated with shrimp alkaline phosphatase and column purified using a Qiagen gel purification kit. The XhoI/KpnI-digested *S. trueperi* dds PCR product was ligated into the prepared vector using T4 DNA ligase for 14-16 hours at 16°C. One μ L of the ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells,

15 which were then plated on LBK (25 μ g/mL) media. Individual colonies were resuspended in about 25 μ L of DI water, and 2 μ L of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μ L of the heated cells was used in a 25 μ L PCR reaction using the SHDDSMCSF and SHDDSMCSR primers. The PCR mix contained the following: 1X

20 Taq PCR buffer, 0.2 μ M each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed under the following conditions: an initial denaturation at 94°C for 2 minutes; 6 cycles of 94°C for 30 seconds, 55°C for 45 seconds, and 72°C for 3 minutes; 25 cycles of 94°C for 30 seconds, 61°C 45 seconds, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. The

25 resulting plasmid containing the Stdss sequence under the control of the rrnB promotor was designated pMCS2rrnBP/Stdss.

The pMCS2rrnBP/Stdss plasmid was electroporated into *E. coli* strain S17-1. Using the S17-1 strain, the pMCS2rrnBP/Stdss plasmid was transferred to *R. sphaeroides* 35053 by conjugation. Individual colonies were purified by restreaking on LBK plates.

30 Single colonies were screened by PCR using the SHDDSMCSF and SHDDSMCSR primers to confirm the presence of the insert as described above.

pMCS2glnBP/Rsdds

The nucleic acid encoding a *R. sphaeroides* polypeptide having DDS activity was cloned in the pMCS2glnBP vector as follows. The *R. sphaeroides* dds gene was PCR amplified using the following primer pair.

RSDDSF 5'-TAGAGAATTCGAAGGAAGAGCATGGGATTGGACG-
AGGTTTC-3' (SEQ ID NO:151)

RSDDSR 5'-TACTACTTGTATGTAGGTACCACTTGCGGTCGGAC-
TGATAG-3' (SEQ ID NO:152)

The forward primer introduced an EcoRI restriction site and a ribosomal binding site that was designed based on *R. sphaeroides* dxs1 gene. The reverse primer introduced a KpnI restriction site. Following reaction mix and PCR program was used to amplify the *R. sphaeroides* dds gene.

Reaction Mix		Program
Pfu 10X buffer	10 µL	94°C 2 minutes
DMSO	5 µL	7 cycles of:
dNTP mix (10 mM)	3 µL	94°C 30 seconds
20 RSDDSF (100 µM)	1 µL	55°C 45 seconds
RSDDSR (100 µM)	1 µL	72°C 3 minutes
Genomic DNA (50 ng/µL)	2 µL	25 cycles of:
Pfu enzyme (2.5 U/µL)	2 µL	94°C 30 seconds
DI water	76 µL	62°C 45 seconds
25		72°C 3 minutes
Total:	100 µL	72°C 7 minutes
		4°C Until used further

The PCR product was separated on a 1% TAE-agarose gel, and a fragment about 1.6 Kb in size was excised. The excised DNA was isolated using a Qiagen gel isolation kit. The isolated DNA was restricted with EcoRI and KpnI and was column purified using a Qiagen gel isolation kit. Three µg of pMCS2glnBP vector DNA was digested with KpnI, and the linear DNA was gel isolated using a Qiagen gel isolation kit. The vector was further digested with EcoRI, and the DNA was column purified. The double-digested vector was then dephosphorylated with shrimp alkaline phosphatase and column

purified using a Qiagen gel purification kit. The KpnI/EcoRI-digested *R. sphaeroides* dds PCR product with the *R. sphaeroides* dxs1 ribosomal binding site described above was ligated into the prepared vector using T4 DNA ligase for 14-16 hours at 16°C. One µL of the ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK (25 µg/mL) media. Individual colonies were resuspended in about 25 µL of DI water, and 2 µL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 µL of the heated cells was used in a 25 µL PCR reaction using the glnBF and RSDDSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 µM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed under the following conditions: an initial denaturation at 94°C for 2 minutes; 6 cycles of 94°C for 30 seconds, 55°C for 45 seconds, and 72°C for 3 minutes; 25 cycles of 94°C for 30 seconds, 62°C 45 seconds, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. A large scale plasmid preparation was done on a culture of a colony containing the Rsdds PCR product, and the glnBP/Rsdds region was sequenced to confirm the lack of nucleotide errors. The resulting plasmid containing the Rsdds sequence under the control of the glnB promoter was designated pMCS2glnBP/Rsdds.

The pMCS2glnBP/Rsdds plasmid DNA was electroporated into electrocompetent *R. sphaeroides* strain 35053 cells as well as electrocompetent carotenoid-deficient mutant cells of 35053 (ATCC 35053/ΔcrtE). Individual colonies of both strains were screened by PCR using the glnBF and RSDDSR primers to confirm the presence of the insert as described above.

25 pMCS2glnBP/Stdds

The nucleic acid encoding a *S. trueperi* polypeptide having DDS activity was cloned in the pMCS2glnBP vector as follows. The *S. trueperi* dds gene was PCR amplified using the following primer pair.

30 SHDDSECOVF 5'-GCGTGATATCGAAGGAAGAGCATGAGCGC-
AACCGTCCACCG-3' (SEQ ID NO:153)

SHDDSKPNR 5'-ACTGCTAGGGTCCGAGGTACCGACATGGACGA-
GGAAGACGC-3' (SEQ ID NO:154)

The forward primer introduced an EcoRV restriction site and a ribosomal binding
5 site that was designed based on the *R. sphaeroides* dxs1 gene. The reverse primer
introduced a KpnI restriction site. The following reaction mix and PCR program were
used to amplify the *S. trueperi* dds gene.

Reaction Mix		Program
10	Pfu 10X buffer 10 μ L	94°C 2 minutes
	DMSO 5 μ L	7 cycles of:
	dNTP mix (10 mM) 3 μ L	94°C 30 seconds
	SHDDSECOVF (100 μ M) 1 μ L	58°C 45 seconds
	SHDDSKPNR (100 μ M) 1 μ L	72°C 3 minutes
15	Genomic DNA (50 ng/ μ L) 2 μ L	25 cycles of:
	Pfu enzyme (2.5 U/ μ L) 2 μ L	94°C 30 seconds
	DI water 76 μ L	65°C 45 seconds
		72°C 3 minutes
	Total: 100 μ L	72°C 7 minutes
20		4°C Until used further

The PCR product was separated on a 1% TAE-agarose gel, and a fragment about
1.2 Kb in size was excised. The excised DNA was isolated using a Qiagen gel isolation
kit. The isolated DNA was restricted with EcoRV and KpnI and was column purified
25 using a Qiagen gel isolation kit. Three μ g of pMCS2glnBP vector DNA was digested
with KpnI, and the linear DNA was gel isolated using a Qiagen gel isolation kit. The
vector was further digested with EcoRV, and the DNA was column purified. The double-
digested vector was then dephosphorylated with shrimp alkaline phosphatase and column
purified using a Qiagen gel purification kit. The KpnI/EcoRV-digested *S. trueperi* dds
30 PCR product with the *R. sphaeroides* dxs1 ribosomal binding site was ligated into the
prepared vector using T4 DNA ligase for 14-16 hours at 16°C. One μ L of the ligation
reaction was transformed into *E. coli* Electromax™ DH10B™ cells, which were plated on
LBK (25 μ g/mL) media. Individual colonies were resuspended in about 25 μ L of DI
water, and 2 μ L of the resuspension was plated on LBK. The remnant resuspension was
35 heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μ L of the heated

cells was used in a 25 μ L PCR reaction using the glnBF and RSDDSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μ M each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed under the following conditions: an initial denaturation at 94°C for 2 minutes; 6 cycles of 94°C for 30 seconds, 58°C for 45 seconds, and 72°C for 3 minutes; 25 cycles of 94°C for 30 seconds, 65°C 45 seconds, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. A large scale plasmid preparation was done on a culture of a colony containing the Stdss PCR product, and the glnBP/Stdss region was sequenced to confirm the lack of nucleotide errors. The resulting plasmid containing the Stdss sequence under the control of the glnB promotor was designated pMCS2glnBP/Stdss.

The pMCS2glnBP/Stdss plasmid DNA was electroporated into electrocompetent cells of *R. sphaeroides* strain 35053 and a carotenoid-deficient mutant of 35053 (ATCC 35053/ Δ crtE). Individual colonies of both strains were screened by PCR using the glnBF and SHDDSKPNR primers to confirm the presence of the insert as described above.

pMCS2tetP/Stdxs

The nucleic acid encoding a *S. trueperi* polypeptide having DXS activity was cloned in the pMCS2tetP vector as follows. The pMCS2tetP plasmid DNA was digested with the restriction enzyme KpnI, cleaned with a QIAquick PCR Purification Kit, and digested with the restriction enzyme ClaI. The enzyme reactions were inactivated by heating at 65°C for 20 minutes. The digested vector DNA was then dephosphorylated with shrimp alkaline phosphatase and gel purified on a 1% TAE-agarose gel. The KpnI/ClaI-digested *S. trueperi* dxs PCR product described above with the *R. sphaeroides* dxs1 ribosomal binding site was ligated into the prepared vector using T4 DNA ligase for 16 hours at 16°C. One μ L of the ligation reaction was transformed into *E. coli* Electromax™ DH5 α ™ cells, which were plated on LBK media. Individual colonies were resuspended in about 25 μ L of 10 mM Tris, and 2 μ L of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μ L of the heated cells was used in a 25 μ L PCR reaction using the SXSClAF2 and SHDXSMCSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μ M each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of

Taq DNA polymerase per reaction. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 54°C for 1 minute, and 72°C for 3.5 minutes; 24 cycles of 94°C for 30 seconds, 60°C 1 minute, and 72°C for 3.5 minutes; and a final extension for 7
5 minutes at 72°C. A large scale plasmid preparation was done on a culture of a colony containing the *S. trueperi* dxs PCR product, and the tetP/Stdxs region was sequenced to confirm the lack of nucleotide errors. The resulting plasmid containing the Stdxs sequence under the control of the tet promotor was designated pMCS2tetP/Stdxs.

Plasmid DNA (pMCS2tetP/Stdxs) was electroporated into electrocompetent cells
10 of *R. sphaeroides* strain 35053 and a carotenoid-deficient mutant of 35053 (ATCC 35053/ Δ crtE). Individual colonies of both strains, along with an *E. coli* control, were screened by PCR using the TETXBAF and STDXSMCSR primers to confirm the presence of the insert as described above.

15 pMCS2tetP/Rsdds

The nucleic acid encoding a *R. sphaeroides* polypeptide having DDS activity was cloned in the pMCS2tetP vector as follows. Three μ g of plasmid DNA of the pMCS2tetP vector was digested with the restriction enzyme KpnI. The digested DNA was cleaned with a QIAquick PCR Purification Kit and digested with the restriction enzyme EcoRI,
20 after which the enzyme was inactivated by heating at 65°C for 20 minutes. The digested vector DNA was then dephosphorylated with shrimp alkaline phosphatase and gel purified. Sixty ng of vector DNA was ligated with 120 ng of the KpnI/EcoR I-digested *R. sphaeroides* dds PCR product described above using T4 DNA ligase at 16°C for 16 hours. One μ L of the ligation reaction was transformed into *E. coli* Electromax™ DH5 α ™,
25 which were then plated on LBK media. Individual colonies were resuspended in about 25 μ L of 10 mM Tris, and 2 μ L of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μ L of the heated cells used in a 25 μ L PCR reaction using the TETXBAF and RSDDSMCSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2
30 μ M each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed in a MJ Research PTC100

under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 55°C for 1 minute, and 72°C for 3 minutes; 24 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. Plasmid DNA was isolated for a colony having the desired insert, and the
5 tetP/Rsdds region was sequenced to confirm the lack of nucleotide errors from PCR. The resulting plasmid containing the Rsdds sequence under the control of the tet promoter was designated pMCS2tetP/Rsdds.

Plasmid DNA (pMCS2tetP/Rsdds) was electroporated into electrocompetent cells of *R. sphaeroides* strain 35053 and the ATCC 35053/ Δ crtE strain. Individual
10 colonies of both strains, along with an *E. coli* control, were screened by PCR using the TETXBAF and RSDDSMCSR primers to confirm the presence of the insert as described above.

pMCS2tetP/Stdds

15 The nucleic acid encoding a *S. trueperi* polypeptide having DDS activity was cloned in the pMCS2tetP vector as follows. Three μ g of pMCS2tetP plasmid DNA was digested with the restriction enzyme KpnI. The digested DNA was gel purified and digested with the restriction enzyme EcoRV. The enzyme was then inactivated by heating at 80°C for 20 minutes, and the DNA dephosphorylated with shrimp alkaline
20 phosphatase. The dephosphorylated DNA was purified using a QIAquick PCR purification kit. Fifty μ g of digested vector DNA was ligated with 150 ng of the KpnI/EcoRV-digested *S. trueperi* dds PCR product described above using T4 DNA ligase at 16°C for 16 hours. One μ L of the ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK media. Individual
25 colonies were resuspended in about 25 μ L of 10 mM Tris, and 2 μ L of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μ L of the heated cells used in a 25 μ L PCR reaction using the TETXBAF and STDDSMCSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μ M each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1
30 unit of Taq DNA polymerase per reaction. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2

minutes; 8 cycles of 94°C for 30 seconds, 55°C for 1 minute, and 72°C for 3 minutes; 24 cycles of 94°C for 30 seconds, 64°C for 1 minute, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. Plasmid DNA was isolated for a colony having the desired insert and was sequenced in the tetP/Stdds region to confirm the DNA sequence of the insert. The resulting plasmid containing the Stdds sequence under the control of the tet promoter was designated pMCS2tetP/Stdds.

Plasmid DNA (pMCS2tetP/Stdds) was electroporated into electrocompetent cells of *R. sphaeroides* strain 35053 and the ATCC 35053/ Δ crtE strain. Individual colonies of both strains, along with an *E. coli* control, were screened by PCR using the TETXBAF and STDDSMCSR primers to confirm the presence of the insert as described above.

pMCS2tetP/Stdxs/Rsdds

Nucleic acid encoding a *S. trueperi* polypeptide having DXS activity as well as nucleic acid encoding a *R. sphaeroides* polypeptide having DDS activity was cloned into the pMCS2tetP vector as follows. A vector containing both the *S. trueperi* dxs gene and the *R. sphaeroides* dds gene, each behind a tet promoter, was constructed using the pMCS2tetP/Stdxs construct described above as the starting vector. This vector was digested with restriction enzyme XbaI, cleaned with a QIAquick PCR Purification Kit, and digested with the restriction enzyme Bpu10I (Fermentas, Hanover, MD). The enzyme reaction was inactivated by heating for 20 minutes at 80°C. The digested vector DNA was then dephosphorylated using shrimp alkaline phosphatase and gel purified on a 1% TAE-agarose gel.

A PCR product containing a tet promoter region followed by a *R. sphaeroides* dds gene was amplified using the pMCS2tetP/Rsdds construct described above as template. The PCR mix contained the following: 1X Native Plus Pfu buffer, 5 ng plasmid template, 0.2 μ M each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 10 units of native Pfu DNA polymerase in a final volume of 200 μ L. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 55°C for 1 minute, and 72°C for 3 minutes; 24 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. The amplification product was then separated by gel

electrophoresis using a 1% TAE-agarose gel. A 1.6 Kb fragment was excised from the gel and purified. The purified fragment was digested with Bpu10I, cleaned with a QIAquick PCR Purification Kit, digested with Xba I restriction enzyme, purified again with a QIAquick PCR Purification Kit, and quantified on a minigel.

- 5 60 ng of the prepared pMCS2tetP/Stdxs vector was ligated with 70 ng of the digested tetP/Rsdds PCR product using T4 DNA ligase at 16°C for 16 hours. One µL of ligation reaction was used to electroporate 40 µL of *E. coli* Electromax™ DH5α™ cells. Electroporated cells were plated on LBK media. Individual colonies were screened by PCR using the RSDDSMCSF and STDXSMSR primers, which produced a 4.1 Kb
- 10 band. Individual colonies were resuspended in about 25 µL of 10 mM Tris, and 2 µL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 µL of the heated cells used in a 25 µL PCR reaction. The PCR reaction mix contained 0.2 µM each primer, 1X Genome Advantage (Clontech, Palo Alto, CA) reaction buffer, 1 M GCMelt, 1.1 mM Mg(OAc)₂,
- 15 0.2 mM each dNTP, and 1X Genome Advantage Polymerase. The PCR was conducted in a MJ Research PTC100 and consisted of an initial denaturation at 94°C for 1.5 minutes; 32 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 60°C, and a 6.5 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes. A large-scale plasmid prep was done for a colony that had the desired insert, and plasmid DNA
- 20 was sequenced through the tetP/Rsdds region to confirm the lack of nucleotide errors from PCR. The resulting plasmid containing the Stdxs sequence under the control of the tet promotor and the Rsdds sequence under the control of the tet promotor was designated pMCS2tetP/Stdxs/Rsdds.

- Plasmid DNA (pMCS2tetP/Stdxs/Rsdds) was electroporated into
- 25 electrocompetent cells of *R. sphaeroides* strains 35053 and the ATCC 35053/ΔcrtE. Individual colonies of both strains, along with an *E. coli* control, were screened by PCR using the RSDDSMCSF and STDDSMCSR primers to confirm the presence of the insert as described above.

pMCS2tetP/Stdxr

Nucleic acid encoding a *S. trueperi* polypeptide having DXR activity was cloned into the pMCS2tetP vector as follows. The *S. trueperi* dxr gene was amplified using genomic DNA as template. The following primers were designed to introduce an EcoRV
 5 restriction site and a ribosomal binding based on *R. sphaeroides* dxs1 gene at the beginning of the amplified fragment and a KpnI site at the end of the amplified fragment.

SXRRVF 5'-GATGATATCGAAGGAAGAGCATGGTGAAGCGCGT-
 CACGGTGT-3' (SEQ ID NO:155)
 10 SXRKPNR 5'-CAAGAGTCAGAAGGTACCCGCCAGAATGGTGAGC-
 AGGATG-3' (SEQ ID NO:156)

The PCR mix contained the following: 1X Native Plus Pfu buffer, 200 ng genomic DNA, 0.2 μ M of each primer, 0.2 mM of each dNTP, 5% DMSO (v/v), and 10
 15 units of native Pfu DNA polymerase in a final volume of 200 μ L. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 59°C for 1 minute, and 72°C for 3 minutes; 24 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 3
 20 minutes; and a final extension for 7 minutes at 72°C. The amplification product was then separated by gel electrophoresis using a 1 % TAE-agarose gel. A 1.0 Kb fragment was excised from the gel and purified. The purified fragment was digested simultaneously with EcoRV and KpnI restriction enzymes, purified with a QIAquick PCR Purification Kit, and checked on a minigel.

Fifty ng of the EcoRV, KpnI-digested pMCS2tetP vector described above for the
 25 pMCS2tetP/Stdss construct was ligated with 75 ng of the digested *S. trueperi* dxr PCR product using T4 DNA ligase at 20°C for 4 hours. One μ L of ligation reaction was used to electroporate 40 μ L of *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK media. Individual colonies were selected and screened by PCR using the TETXBAF and SXRKPNR primers. The PCR mix contained the following: 1X Taq PCR
 30 buffer, 200 ng genomic DNA, 0.2 μ M of each primer, 0.2 mM of each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per 25 μ L reaction. The PCR reaction was

performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 32 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. A large-scale plasmid preparation was done for a colony that had the desired insert, and the tetP/Stdxr region
5 was sequenced to confirm the DNA sequence of the insert. The resulting plasmid containing the Stdxr sequence under the control of the tet promotor was designated pMCS2tetP/Stdxr.

Plasmid DNA (pMCS2tetP/Stdxr) was electroporated into electrocompetent cells of *R. sphaeroides* strains 35053 and ATCC 35053/ Δ crtE. Individual colonies of both
10 strains, along with an *E. coli* control, were screened by PCR using the TETXBAF and SXRKPNR primers to confirm the presence of the insert as described above.

pMCS2tetP/Stdxr/Stdds

Nucleic acid encoding a *S. trueperi* polypeptide having DXR activity as well as
15 nucleic acid encoding a *S. trueperi* polypeptide having DDS activity was cloned into the pMCS2tetP vector as follows. A vector containing both the *S. trueperi* dxr and dds genes, each behind a tet promoter, was constructed using the pMCS2tetP/Stdds construct described above as the starting vector. This vector was digested with restriction enzyme XbaI, cleaned with a QIAquick PCR Purification Kit, and digested with the restriction
20 enzyme Bpu10I (Fermentas). The enzyme reaction was inactivated by heating for 20 minutes at 80°C. The digested vector DNA was then dephosphorylated with shrimp alkaline phosphatase and gel purified.

A PCR product containing a tet promoter region followed by a *S. trueperi* dxr gene was amplified using the pMCS2tetP/Stdxr construct described above as template
25 and primers TETBPUF and SXRXBAR. The SXRXBAR primer, having the following sequence, was designed to introduce an XbaI restriction site on the end of the PCR product.

SXRXBAR 5'-CAAGAGTCAGAATCTAGACGCCAGAATGGTGA-
30 GCAGGATG-3' (SEQ ID NO:157)

The PCR mix contained the following: 1X Native Plus Pfu buffer, 5 ng plasmid template, 0.2 μ M each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 10 units of native Pfu DNA polymerase in a final volume of 200 μ L. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial
5 denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 59°C for 1 minute, and 72°C for 3.5 minutes; 24 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 3.5 minutes; and a final extension for 7 minutes at 72°C. The amplification product was then separated by gel electrophoresis using a 1% TAE-agarose gel. A 1.4 Kb fragment
10 was excised from the gel and purified. The purified fragment was digested with Bpu10I, cleaned with a QIAquick PCR Purification Kit, digested with XbaI restriction enzyme, purified again with a QIAquick PCR Purification Kit, and quantified on a minigel.

Sixty ng of the prepared pMCS2tetP/Stdds vector was ligated with 80 ng of the digested tetP/Stdxr PCR product using T4 DNA ligase at 16°C for 16 hours. One μ L of ligation reaction was used to electroporate 40 μ L of *E. coli* Electromax™ DH10B™ cells,
15 which were then plated on LBK media. Individual colonies were screened by PCR using the SXREVF and SDSKPNR primers. Colonies were resuspended in about 25 μ L of 10 mM Tris, and 2 μ L of the resuspension was plated on LBK media. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μ L of the heated cells used in a 25 μ L PCR reaction. The PCR mix contained the
20 following: 1X Taq PCR buffer, 0.2 μ M each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 58°C for 1 minute, and 72°C for 4.5 minutes; 24 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 4.5 minutes; and
25 a final extension for 7 minutes at 72°C. A large-scale plasmid preparation was done for a colony that had the desired insert, and the tetP/Stdxr region was sequenced to confirm the lack of nucleotide errors from PCR. The resulting plasmid containing the Stdxr sequence under the control of the tet promotor and the Stdds sequence under the control of the tet promotor was designated pMCS2tetP/Stdxr/Stdds.

30 Plasmid DNA (pMCS2tetP/Stdxr/Stdds) was electroporated into electrocompetent cells of *R. sphaeroides* strains 35053 and ATCC 35053/ Δ crtE. Individual colonies of

both strains, along with an *E. coli* control, were screened by PCR using the SXREVF and SDSKPNR primers to confirm the presence of the insert as described above.

pMCS2tetP/EcUbiC

5 Nucleic acid encoding a *E. coli* polypeptide having chorismate lyase activity was cloned into the pMCS2tetP vector as follows. The *E. coli* ubiC gene was amplified using genomic DNA from *E. coli* strain DH10B as template. The following primers were designed to introduce an EcoRV restriction site and a ribosomal binding site based on *R. sphaeroides* dxs1 gene at the beginning of the amplified fragment, and a KpnI site at the
10 end of the amplified fragment.

UBICRVF 5'-CTAGATATCGGAAGGAAGAGCATGTCACAC-
CCCGCGTTA-3' (SEQ ID NO:158)

UBICKPNR 5'-TCAGGTACCGTGTCGCCACCCACAACGCC-
15 CATAATG-3' (SEQ ID NO:159)

The PCR mix contained the following: 1X Native Plus Pfu buffer, 200 ng genomic DNA, 0.2 μ M each primer, 0.2 mM each dNTP, and 10 units of native Pfu DNA polymerase in a final volume of 200 μ L. The PCR reaction was performed in a MJ
20 Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 57°C for 1 minute, and 72°C for 2.5 minutes; 24 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 2.5 minutes; and a final extension for 7 minutes at 72°C. The amplification product was then separated by gel electrophoresis using a 1.5 % TAE-agarose gel. A 650 bp fragment was excised from the
25 gel and purified. The purified fragment was digested with EcoRV, cleaned with a QIAquick PCR Purification Kit, digested with KpnI restriction enzyme, purified again with a QIAquick PCR Purification Kit, and quantified on a minigel.

Seventy-five ng of the EcoRV, KpnI-digested pMCS2tetP vector described above for the pMCS2tetP/Stdds construct was ligated with 70 ng of the digested ubiC PCR
30 product using T4 DNA ligase at 16°C for 16 hours. One μ L of ligation reaction was used to electroporate 40 μ L of *E. coli* Electromax™ DH5 α ™ cells, which were then plated on

LBK media. Individual colonies were resuspended in about 25 μ L of 10 mM Tris, and 2 μ L of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μ L of the heated cells used in a 25 μ L PCR reaction using the TETXBAF and UBICKPNR primers. The PCR mix contained
5 the following: 1X Taq PCR buffer, 0.2 μ M each primer, 0.2 mM each dNTP, and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 32 cycles of 94°C for 30 seconds, 62°C for 1 minute, and 72°C for 2 minutes; and a final extension for 7 minutes at 72°C. A large-scale plasmid preparation was done
10 for a colony that had the desired insert and the tetP/ubiC region was sequenced to confirm the DNA sequence of the insert. The resulting plasmid containing the UbiC sequence under the control of the tet promotor was designated pMCS2tetP/EcUbiC.

Plasmid DNA (pMCS2tetP/EcUbiC) was electroporated into electrocompetent cells of *R. sphaeroides* strain 35053 and the ATCC 35053/ Δ crtE strain. Individual
15 colonies of both strains, along with an *E. coli* control, were screened by PCR using the TETXBAF and UBICKPNR primers to confirm the presence of the insert as described above with the addition of 5% DMSO (v/v) to the PCR reaction.

pMCS2tetP/Stdxs/Rsdds/EcUbiC

20 Nucleic acid encoding an *S. trueperi* polypeptide having DXS activity, nucleic acid encoding an *R. sphaeroides* polypeptide having DDS activity, and nucleic acid encoding an *E. coli* polypeptide having chorismate lyase activity was cloned into the pMCS2tetP vector as follows. A vector containing the *S. trueperi* dxs gene, the *R. sphaeroides* dds gene, and the *E. coli* ubiC gene, each behind a tet promoter, was
25 constructed using the pMCS2tetP/Stdxs/Rsdds construct described above as the starting vector. This vector was digested with restriction enzyme KpnI, cleaned with a QIAquick PCR Purification Kit, and digested with the restriction enzyme NsiI. The enzyme reaction was inactivated by heating for 20 minutes at 65°C. The digested vector DNA was then dephosphorylated with shrimp alkaline phosphatase and gel purified.

30 A PCR product containing a tet promoter region followed by an *E. coli* ubiC gene was amplified using the pMCS2tetP/EcUbiC construct described above as template. The

following primers were designed to introduce an KpnI restriction site at the beginning of the amplified fragment and an NsiI site at the end of the amplified fragment.

5 TETKPNF 5'-TAGGGTACCACCGTCTACGCCGACCT-
CGTTCAAC-3' (SEQ ID NO:160)
UBICNSIR 5'-TGTATGCATGTCGCCACCCACAACGC-
CCATAATG-3' (SEQ ID NO:161)

10 The PCR mix contained the following: 1X Native Plus Pfu buffer, 5 ng plasmid template, 0.2 μ M each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 10 units of native Pfu DNA polymerase in a final volume of 200 μ L. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 62°C for 1 minute, and 72°C for 2.5 minutes; 24 cycles of 94°C for 30 seconds, 66°C 1 minute, and 72°C for 15 2.5 minutes; and a final extension for 7 minutes at 72°C. The amplification product was then separated by gel electrophoresis using a 1% TAE-agarose gel. An 850 bp fragment was excised from the gel and purified. The purified fragment was digested with the restriction enzyme NsiI, cleaned with a QIAquick PCR Purification Kit, digested with the restriction enzyme KpnI, purified again with a QIAquick PCR Purification Kit, and 20 quantified on a minigel.

Fifty ng of the prepared pMCS2tetP/Stdxs/Rsdds vector was ligated with 35 ng of the digested tetP/ubiC PCR product using T4 DNA ligase at 16°C for 16 hours. One μ L of ligation reaction was used to electroporate 40 μ L of *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK media. Individual colonies were resuspended in 25 25 μ L of 10 mM Tris, and 2 μ L of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μ L of the heated cells used in a 25 μ L PCR reaction using the SXSClaf2 and UBICNSIR primers. The PCR reaction mix contained 1X GC-RICH PCR reaction buffer, 1.0 M GC-RICH resolution solution, 0.2 μ M each primer, 0.2 mM each dNTP, 30 and 1 unit of GC-RICH enzyme mix per reaction (Roche). The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial

denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 60°C for 1 minute, and 72°C for 5 minutes; 24 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 5 minutes; and a final extension for 7 minutes at 72°C. A large-scale plasmid preparation was done for a colony that had the desired insert, and plasmid DNA was sequenced through the tetP/ubiC region to confirm the lack of nucleotide errors from PCR. The resulting plasmid containing Stdxs sequence under the control of the tet promotor, the Rsdds sequence under the control of the tet promotor, and the UbiC sequence under the control of the tet promotor was designated pMCS2tetP/Stdxs/Rsdds/EcUbiC.

Plasmid DNA (pMCS2tetP/Stdxs/Rsdds/EcUbiC) was electroporated into electrocompetent cells of *R. sphaeroides* strains 35053 and ATCC 35053/ Δ crtE. Individual colonies of both strains, along with an *E. coli* control, were screened by PCR using the SXSClAF2 and UBICNSIR primers to confirm the presence of the insert as described above.

15 pMCS2tetP/RsLytB

Nucleic acid encoding a LytB *R. sphaeroides* polypeptide was cloned into the pMCS2tetP vector as follows. The *R. sphaeroides* lytB was identified by TBLASTN analysis of its genome using an *E. coli* lytB sequence as a query. Based on the identified sequence the following primers were designed to PCR amplify the gene:

LYTBHINDF 5'-GACGAAGCTTGAAGGAAGAGCATGCCTCCCCTCA-
CCCTCTATC-3' (SEQ ID NO:162)

LYTBKPNR 5'-GTCAGTGAATGAATGGTACCGCAGCCGAGAACCG-
CCAGAAGCC-3' (SEQ ID NO:163)

25

The primers introduced a HindIII restriction site and ribosomal binding site at the 5' end, and a KpnI restriction site at the 3' end. The following reaction mix and PCR program were used to amplify the lytB gene.

30	Reaction Mix	Program
	Pfu 10X buffer 10 μ L	94°C 2 minutes
	DMSO 5 μ L	7 cycles of:

	dNTP mix (10 mM)	3 μ L	94°C	30 seconds
	LYTBHINDF (100 μ M)	1 μ L	59°C	45 seconds
	LYTBKPNR (100 μ M)	1 μ L	72°C	3 minutes
	Genomic DNA (50 ng/ μ L)	2 μ L	25 cycles of:	
5	Pfu enzyme (2.5 U/ μ L)	2 μ L	94°C	30 seconds
	DI water	76 μ L	66°C	45 seconds
			72°C	3 minutes
	Total:	100 μ L	72°C	7 minutes
			4°C	Until used further

10

The PCR product was run on a 1% TAE-agarose gel, and a fragment about 1.1 Kb in size was excised. The excised DNA was isolated using a Qiagen gel isolation kit. The isolated DNA was restricted with HindIII and KpnI, and was column purified using a Qiagen gel isolation kit. Two μ g of pMCS2tetP vector DNA was digested with HindIII, and the linear DNA was gel isolated using a Qiagen gel isolation kit. The vector was further digested with KpnI, and the DNA was column purified. The double-digested vector was then dephosphorylated with shrimp alkaline phosphatase and column purified using a Qiagen gel purification kit. The KpnI/HindIII-digested *R. sphaeroides* lytB PCR product with the *R. sphaeroides* dxs1 ribosomal binding site described above was ligated into the prepared vector using T4 DNA ligase for 14-16 hours at 16°C. One μ L of the ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK (25 μ g/mL) media. Individual colonies were resuspended in about 25 μ L of DI water, and 2 μ L of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μ L of the heated cells was used in a 25 μ L PCR reaction using the LYTBHINDF and LYTBKPNR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μ M each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 59°C for 1 minute, and 72°C for 3 minutes; 24 cycles of 94°C for 30 seconds, 66°C for 1 minute, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. A large scale plasmid preparation was done on a culture of a colony containing the lytB PCR product, and the tetP/lytB region was sequenced to confirm the lack of nucleotide errors.

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The resulting plasmid containing the RsLytB sequence under the control of the tet promoter was designated pMCS2tetP/RsLytB.

Plasmid DNA (pMCS2tetP/RsLytB) was electroporated into electrocompetent cells of *R. sphaeroides* strain 35053 and a carotenoid-deficient mutant of 35053 (ATCC 35053/ Δ crtE). Individual colonies of both strains, along with an *E. coli* control, were screened by PCR using the TETXBAF and LYTBKPNR primers to confirm the presence of the insert as described above.

pMCS2tetP/Stdxs/Rsdds/RsLytB

Nucleic acid encoding an *S. trueperi* polypeptide having DXS activity, nucleic acid encoding an *R. sphaeroides* polypeptide having DDS activity, and nucleic acid encoding LytB from *R. sphaeroides* were cloned into the pMCS2tetP vector as follows. The *R. sphaeroides* lytB gene was cloned and expressed along with the *R. sphaeroides* dds and *S. trueperi* dxs genes. In this triple expression system, each gene was expressed through its own tetP. The *R. sphaeroides* lytB gene was PCR amplified along with the tetP using the following primers.

TETKPNF 5'-TAGGGTACCACCGTCTACGCCGACCTC-
GTTGAAC-3' (SEQ ID NO:164)

LYTBNSIR 5'-AGGCAATGCATGCAGCCGAGAACCGCC-
AGAAGCC-3' (SEQ ID NO:165)

The following PCR mix and program were used to PCR amplify the lytB gene along with the tetP.

25

Reaction Mix		Program
Pfu 10X buffer	10 μ L	94°C 2 minutes
DMSO	5 μ L	7 cycles of:
dNTP mix (10 mM)	3 μ L	94°C 30 seconds
TETKPNF (100 μ M)	1 μ L	63°C 45 seconds
LYTBNSIR (100 μ M)	1 μ L	72°C 3 minutes
pMCS2tetP/lytB (10 ng/ μ L)	1 μ L	25 cycles of:
Pfu enzyme (2.5 U/ μ L)	2 μ L	94°C 30 seconds
DI water	77 μ L	69°C 45 seconds

30

		72°C	3 minutes
Total:	100 µL	72°C	7 minutes
		4°C	Until used further

5 In this PCR reaction, pMCS2tetP/RsLytB plasmid DNA was used as a template. The PCR product was separated on a 1% TAE-agarose gel, and a fragment about 1.4 Kb in size was excised. The excised DNA was isolated using a Qiagen gel isolation kit. The isolated DNA was restricted with NsiI and KpnI, and was column purified using a Qiagen gel isolation kit. Two µg of pMCS2tetP/Stdxs/Rsdds plasmid DNA was digested with

10 NsiI, and the linear DNA was gel isolated using a Qiagen gel isolation kit. The vector was further digested with KpnI, and the DNA was column purified. The double-digested vector was then dephosphorylated with shrimp alkaline phosphatase and column purified using a Qiagen gel purification kit. The KpnI/NsiI-digested PCR product was ligated into the prepared plasmid using T4 DNA ligase for 14-16 hours at 16°C. One µL of the

15 ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK (25 µg/mL) media. Individual colonies were resuspended in about 25 µL of DI water, and 2 µL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 µL of the heated cells was used in a 25 µL PCR reaction using the SXSClaf2 and

20 LYTBNSIR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 µM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed under the following conditions: an initial denaturation at 94°C for 2 minutes; 6 cycles of 94°C for 30 seconds, 59°C for 45 sec, and 72°C for 4 minutes; 25 cycles of 94°C for 30 seconds, 65°C for 45 seconds, and 72°C for

25 4 minutes; and a final extension for 7 minutes at 72°C. A large scale plasmid preparation was done on a culture of a colony containing the correct insert, and the tetP/lytB region was sequenced to confirm the lack of nucleotide errors. The resulting plasmid containing Stdxs sequence under the control of the tet promoter, the Rsdds sequence under the control of the tet promoter, and the LytB sequence under the control of the tet promoter

30 was designated pMCS2tetP/Stdxs/Rsdds/RsLytB.

Plasmid DNA (pMCS2tetP/Stdxs/Rsdds/RsLytB) was electroporated into electrocompetent cells of *R. sphaeroides* strain 35053 and a carotenoid-deficient mutant

of 35053 (ATCC 35053/ Δ crtE). Individual colonies of both strains were screened by PCR using the SXSCLAF2 and LYTBNSIR primers to confirm the presence of the insert as described above.

5 Example 9 – Making recombinant microorganisms containing knock-outs

Various nucleic acid sequences within the *R. sphaeroides* genome were knocked out. All restriction enzymes and T4 DNA ligase were obtained from New England Biolabs (Beverly, MA) unless otherwise indicated. All plasmid DNA preparations were done using QIAprep Spin Miniprep Kits or Qiagen Maxi Prep Kits, and all gel
10 purifications were done using QIAquick Gel Extraction Kits (Qiagen, Valencia, CA).

ATCC 35053/ Δ crtE(kan)

R. sphaeroides cells lacking crtE were made by inserting a kanamycin resistance gene into the crtE sequence as follows. In general, the crtE gene from *R. sphaeroides* was
15 cloned into a pUC19 vector, and a kanamycin gene (kan) was inserted into the gene to inactivate it. The crtE-kan insert was amplified by PCR and cloned into pSUP203, a mobilizable ColE1-based plasmid that is not maintained in *R. sphaeroides* unless it is integrated into a *R. sphaeroides* replicon. This plasmid was transformed into *E. coli* strain S17-1, a strain that is able to mobilize oriT-containing plasmids in conjugations
20 with a second bacterial strain. The S17-1 strain was conjugated with *R. sphaeroides* strain 35053, and colonies were identified in which the crtE-kan insert had replaced the native crtE gene.

The crtE gene from *R. sphaeroides* strain 17023 was amplified by PCR using primers designed to introduce an SphI restriction site at the beginning of the amplified
25 fragment and an XbaI restriction site at the end of the amplified fragment. The sequences of the primers were as follows.

CRTESPHF 5'-AAGCATGCGAAAAAGTTGACACCTGTGGAGTC-3' (SEQ ID NO:166)

30 CRTEXBAR 5'-ACTCTAGAAGCACCTGCGAATGGACGAAG-3' (SEQ ID NO:167)

The fragment amplified included the crtE gene along with 85 nucleotides upstream of the translational start codon and 228 nucleotides downstream of the translational stop codon. The PCR reaction mix contained 0.2 μ M each primer, 1X GC Genomic PCR Buffer (Clontech, Palo Alto, CA), 1 M GC-Melt, 1.1 mM Mg(OAc)₂, 0.2 mM each dNTP, 1X Advantage-GC Genomic Polymerase Mix, and 1 ng of genomic DNA per μ L of reaction mix. The PCR was conducted in a Perkin Elmer Geneamp 2400 and consisted of an initial denaturation at 94°C for 30 seconds; 35 cycles of a 15 second denaturation at 94°C, a one minute annealing at 55°C, and a 3 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes. Fifty μ L of PCR product was separated on a 1% Tris-Acetate-EDTA (TAE)-agarose gel. A 1180 bp fragment was gel purified, and the purified DNA was digested with XbaI and SphI restriction enzymes (Promega, Madison, WI).

pUC19 vector was digested with the restriction enzymes SphI and XbaI, and gel purified on a 1% TAE- agarose gel. Fifty ng of purified vector was ligated with about 150 ng of digested crtE PCR product for 16 hours at 14°C using T4 DNA ligase (Roche Molecular Biochemicals, Indianapolis, IN). One μ L of ligation reaction was transformed into ElectroMAX™ DH10B™ cells (Life Technologies, Gaithersburg, MD), which were then plated on LB media containing 100 μ g/mL ampicillin and 50 μ g/mL of 5-Bromo-4-Chloro-3-Indolyl-B-D-Galactopyranoside (LBKX). Individual, white colonies were resuspended in about 20 μ L of 10 mM Tris, and 2 μ L of the resuspension was plated on LBKX media. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μ L of the heated cells was used in a 25 μ L PCR reaction using the CRTESPHF and CRTEXBAR primers. The PCR reaction mix contained 0.2 μ M each primer, 1X GC Genomic PCR Buffer, 1 M GCMelt, 1.1 mM Mg(OAc)₂, 0.2 mM each dNTP, and 1X Advantage-GC Genomic Polymerase Mix. The PCR was conducted in a Perkin Elmer Geneamp 2400 and consisted of an initial denaturation at 94°C for 30 seconds; 35 cycles of a 15 second denaturation at 94°C, a one minute annealing at 55°C, and a 3 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes. Plasmid DNA was isolated for colonies having a crtE gene insert and was digested with the restriction enzyme HindIII and with a mixture of SphI and XbaI to confirm vector structure.

One μ g of the pUC19crtE construct was digested with XhoI and StuI restriction enzymes. These enzymes cut a 273 bp fragment of DNA from the center of the crtE gene. The digested DNA was separated on a 1% TAE-agarose gel. A 3.6 Kb fragment representing pUC19 and the remaining ends of the crtE gene was excised and purified.

- 5 The kanamycin resistance gene was amplified by PCR from the PCRII vector (Invitrogen, Carlsbad, CA) using primers designed to introduce an StuI restriction site at the beginning of the amplified fragment and an XhoI restriction site at the end of the amplified fragment. The sequences of the primers were as follows.

- 10 KANSTUF 5'-ATAAAGGCCTTACATGGCGATAGCTAGACTG-3' (SEQ ID NO:168)
KANXHOR 5'-AAGGCTCGAGAAGGATCTTACCGCTGTTGAG-3' (SEQ ID NO:169)

- 15 The PCR reaction mix contained 0.2 μ M each primer, 1X Pfu reaction buffer (Stratagene, La Jolla, CA), 0.2 mM each dNTP, 8 units Pfu, and 5 ng of the PCRII vector in a 200 μ L reaction. The PCR was conducted in a Perkin Elmer Geneamp 2400 and consisted of an initial denaturation at 94°C for 2 minutes; 8 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 55°C, and a 2.5 minute extension at 72°C;
20 24 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 55°C, and a 2.5 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes. The PCR product was separated on a 1% TAE- agarose gel, and a 1.2 Kb fragment was excised and purified. One μ g of purified DNA was digested with XhoI and StuI restriction enzymes and cleaned using a QIAquick PCR Purification Kit.

- 25 Fifty ng of the digested pUC19crtE vector DNA was ligated with 75 ng of the digested kan PCR product for 16 hours at 14°C using T4 DNA ligase (Roche). One μ L of ligation mix was electroporated into 40 μ L of *E. coli* ElectroMAX™ DH10B™ electrocompetent cells, which were then plated on LB media containing 100 μ g/mL ampicillin and 50 μ g/mL kanamycin (LBAK). Plasmid DNA was isolated from cultures
30 of individual colonies and was digested in separate reactions with the restriction enzymes PstI, SphI, and a StuI/XbaI mixture to confirm correct vector structure.

The crtE gene with the inserted kan gene was amplified by PCR using primers designed to have ScaI restriction sites on both ends of the fragment. The sequences of the primers were as follows.

- 5 CRTESCAF 5'-ATAGTACTGAAAAAGTTGACACCTGTGGAGTC-3' (SEQ ID NO:170)
CRTESCAR 5'-ATAGTACTAGCACCTGCGAATGGACGAAG-3' (SEQ ID NO:171)

- The PCR reaction mix contained 0.2 μ M each primer, 1X GC Genomic PCR Buffer, 1 M GCMelt, 1.1 mM Mg(OAc)₂, 0.2 mM each dNTP, 1X Advantage-GC Genomic Polymerase Mix, and 1 ng of plasmid DNA per μ L of reaction mix. The PCR was conducted in a Perkin Elmer Geneamp 9600 and consisted of an initial denaturation at 94°C for 1 minute; 8 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 55°C, and a 4 minute extension at 72°C; 25 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 60°C, and a 4 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes. 200 μ L of PCR product was separated on a 1% TAE-agarose gel. A 2.0 Kb fragment was excised and purified. One μ g of purified DNA was digested with ScaI restriction enzyme, and the digested DNA was purified using a QIAquick PCR Purification Kit.
- 20 2.3 μ g of pSUP203 plasmid DNA was digested with ScaI restriction enzyme. The digested DNA was separated on a 1% TAE-agarose gel, and a 7.6 Kb fragment was excised and purified. The purified plasmid DNA was then dephosphorylated using calf intestinal alkaline phosphatase (Promega). 75 ng of dephosphorylated plasmid DNA was ligated with 60 ng and 120 ng of the ScaI-digested crtE-kan PCR product for 16 hours at 25 14°C using T4 DNA ligase (New England BioLabs). One μ L of ligation mix was electroporated into 40 μ L of *E. coli* ElectroMAX™ DH10B™ electrocompetent cells, which were then plated on LB media containing 10 μ g/mL tetracycline, to which pSUP203 carries a resistance gene, and 25 μ g/mL kanamycin. Plasmid DNA was isolated from cultures of individual colonies and digested with ScaI restriction enzyme to check 30 insert size. 100 ng of plasmid DNA derived from a confirmed colony was electroporated into electrocompetent cells of the *E. coli* strain S17-1. This strain contains a

chromosomal copy of the trans-acting elements that mobilize oriT-containing plasmids during conjugation with a second bacterial strain. It also carries a gene conferring resistance to the antibiotics streptomycin and spectinomycin. The transformation reaction was plated on LB media with 10 µg/mL tetracycline, 25 µg/mL kanamycin, and 25 µg/mL streptomycin. Individual colonies were resuspended in about 20 µL of 10 mM Tris and heated for 10 minutes at 95°C to break open the bacterial cells. Two µL of the heated cells was used in a 25 µL PCR reaction using the CRTESCAF and CRTESCAR primers to confirm the presence of the crtE-kan insert. The PCR reaction mix contained 0.2 µM each primer, 1X GC Genomic PCR Buffer, 1.0 M GCMelt, 1.1 mM Mg(OAc)₂, 0.2 mM each dNTP, and 1X Advantage-GC Genomic Polymerase Mix. The PCR was conducted in a Perkin Elmer Geneamp 9600 and consisted of an initial denaturation at 94°C for 1 minute; 30 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 56°C, and a 4 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes.

The pSUP203crtE-kan construct was introduced into *R. sphaeroides* strain 35053 through conjugation with the *E. coli* S17-1 strain carrying this vector. The S17-1 donor was grown in LB media with 25 µg/mL kanamycin and 25 µg/mL streptomycin at 37°C for 16 hours. A growing culture of *R. sphaeroides* strain 35053 was used to inoculate Sistrom's media using 1/5 to 1/10 dilutions, and the subcultures were grown at 30°C for about 20 hours. For both the S17-1crtE-kan and 35053 genotypes, cells were pelleted from 1.5 mL of culture. Pellets were resuspended and pelleted four times in either 1X Sistrom's salts for the 35053 cells or LB media for the S17-1 cells. The pellets were each resuspended in 1.5 mL of LB, and 200 µL of the S17-1 cells was combined with 1.3 mL of the 35053 cells. This mixture was pelleted, the supernatant removed, and the pellet resuspended in 20 µL of LB media. The resuspended cells were spotted onto an LB plate and incubated at 30°C for 7.5 hours. The cells were then scraped off the plate, resuspended in 1.5 mL of 1X Sistrom's salts, and plated (200 µL/plate) on Sistrom's media supplemented with 25 µg/mL kanamycin and 10 µg/mL of telluride (SisKTell). The telluride retards the growth of *E. coli* cells but is detoxified by *R. sphaeroides*. After 7 days, small black colonies were picked off the plates and streaked to fresh plates of the same media. After 6 days of growth, grayish colonies were patched to LB plates containing 25 µg/mL kanamycin (LBK25) and also to LB plates containing 0.75 µg/mL

tetracycline. Desirable double-crossover events, in which the crtE-kan gene was integrated and retained in the genome while the vector DNA was lost, exhibited kanamycin resistance but lacked tetracycline resistance. Colonies resulting from undesirable single-crossover events demonstrated both kanamycin and tetracycline
5 resistance.

The mutants were confirmed using PCR and Southern hybridization as follows. Colonies that exhibited kanamycin resistance, lacked tetracycline resistance, and had a gray phenotype were screened by PCR for the crtE locus using the CRTESCAF and CRTESCAR primers as described above. To confirm that they were *R. sphaeroides*
10 colonies with a truncated crtE gene rather than *E. coli* colonies carrying the vector, colonies were also screened using primers specific to the *R. sphaeroides* ppsR gene and the *E. coli* dxs gene. Individual colonies were resuspended in about 20 µL of 10 mM Tris, and heated for 10 minutes at 95°C to break open the bacterial cells. Two µL of the heated cells were used per 25 µL PCR reaction. The PCR reaction mix contained 0.2 µM
15 each primer, 1X GC Genomic PCR Buffer, 1.0 M GCMelt, 1.1 mM Mg(OAc)₂, 0.2 mM each dNTP, and 1X Advantage-GC Genomic Polymerase Mix. The PCR was conducted in a Perkin Elmer Geneamp 9600 and consisted of an initial denaturation at 94°C for 1 minute; 8 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 55°C, and a 3.5 minute extension at 72°C; 22 cycles of a 30 second denaturation at 94°C, a 1 minute
20 annealing at 61°C, and a 3.5 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes. All suspected 35053crtE-kan colonies produced a crtE band the same size as the S17-1crtE-kan control. They all also produced a band of the expected size for the ppsR gene and did not produce a band for the *E. coli* dxs gene.

To further confirm the presence of double-crossover events, Southern
25 hybridization was conducted on eight 35053crtE-kan colonies as well as *R. sphaeroides* strains 35053 and 17023. Sequence data for the photosynthetic operon of strain 17023 is available in Genbank and was used to determine restriction enzymes likely to have hybridization patterns that would distinguish mutants from non-mutants. Genomic DNA was isolated from each line using a Gentra Puregene DNA Isolation Kit (Gentra,
30 Minneapolis, MN). Two µg of genomic DNA was used in digests with the restriction enzymes ApaI and XhoI. The digests were separated on a 0.8% TAE agarose gel, and the

DNA transferred to a nylon membrane. DIG-labeled molecular weight markers II and III (Roche) were also included on the gel/membrane. DIG-labeled probes of the *crtE* locus were synthesized using a PCR DIG Probe Synthesis Kit (Roche). After baking, membranes were prehybridized in EasyHyb Buffer (Roche) for at least 2 hours and
5 hybridized overnight using 400 nL of a 0.5 DIG labeling reaction per mL of hybridization solution. Detection was conducted using a Wash and Block Buffer Set (Roche). Membranes were washed two times for 5-10 minutes each at room temperature in 2X SSC/0.1% SDS and two times for 15-20 minutes each at 68°C in 0.1X SSC/0.1% SDS. They were then covered with blocking buffer and placed on a shaker for an hour at room
10 temperature. The blocking buffer was replaced with fresh blocking buffer containing 150 mU of AP conjugate per mL of buffer, and the membranes shaken at room temperature for an additional 30 minutes. Membranes were then washed twice for 15 minutes each at room temperature with washing buffer, followed by a five minute wash with detection buffer. The detection buffer was replaced with fresh detection buffer containing 20 µL of
15 NBT/BCIP solution per mL of buffer. This was placed in the dark at room temperature with no shaking until color developed, after which the buffer was replaced with 10 mM Tris-1 mM EDTA solution.

In the *ApaI* digest, the mutant lines exhibited a band of about 850 bp larger than the strain 35053 control, which is the size difference expected from the insertion of the
20 kanamycin gene product in the *StuI/XhoI* sites. For the *XhoI* digest, strain 35053 exhibited a band of about 700 bp, strain 17023 had a band of about 1100 bp, mutant 7C had a band of 1550 bp, and the remaining mutants had a band of 2050 bp. The reason for the size difference in the *XhoI* bands for the mutants was unclear, but mutant 7C was used in further studies due to its possession of the expected band size relative to strain
25 35053. The resulting *R. sphaeroides* mutant containing a *crtE* knockout was designated ATCC 35053/Δ*crtE*(kan).

ATCC 35053/Δ*crtE*

R. sphaeroides cells lacking *crtE* were made using *sacB* selection as follows. A
30 truncated *crtE* gene was cloned into the vector pL01, which is a suicide vector in *R. sphaeroides*. The pL01 vector carries a kanamycin resistance gene, a *B. subtilis sacB*

gene, an oriT sequence, a ColEI replicon, and a multiple cloning site (Lenz *et al.*, *J. Bacteriol.*, 176(14):4385-93 (1994)). The pL01crtE plasmid was introduced into *R. sphaeroides* strain 35053 through conjugation with an *E. coli* donor. The kanamycin resistance gene was used to select for single-crossover events between the truncated crtE

5 gene and the genomic crtE gene that resulted in incorporation of the pL01crtE DNA into the genome. The presence of the sacB gene on the vector allowed for subsequent selection for the loss of the vector DNA from the genome, as expression of this gene in the presence of sucrose is lethal to *E. coli* and to *R. sphaeroides* under certain growth conditions. A portion of the double-crossover events that led to loss of the sacB gene

10 contained the truncated crtE allele. This method of gene knockout is useful because no residual antibiotic resistance gene is left in the genome.

A three-step PCR process was used to create a 249 bp in-frame deletion in the crtE gene. The crtE gene from *R. sphaeroides* strain 35053 was amplified by PCR using primers designed to introduce an SphI restriction site at the beginning of the amplified

15 fragment and a SacI restriction site at the end of the amplified fragment. The sequences of the primers were as follows.

CRTESPHF 5'-CGTGGCATGCGTGTAAGAAAAAGTTGACA-
CCTGTGGAGTC-3' (SEQ ID NO:172)

20 CRTESACR 5'-CTAAGAGCTCAGTTCGGGCTCGGTCTCGC-
CTTTCAGGAAG-3' (SEQ ID NO:173)

The PCR reaction mix contained 0.2 µM each primer, 1X Genome Advantage reaction buffer, 1 M GCMelt, 1.1 mM Mg(OAc)₂, 0.2 mM each dNTP, 1X Genome

25 Advantage Polymerase, and 1 ng of genomic DNA per µL of reaction mix. The PCR was conducted in a Perkin Elmer Geneamp 2400 and consisted of an initial denaturation at 94°C for 2 minutes; 32 cycles of a 30 second denaturation at 94°C, a 45 second annealing at 64°C, and a 3 minute extension at 72°C, followed by a final extension at 72°C for 7 minutes. 200 µL of PCR product was separated on a 1% TAE-agarose gel, and a 1.5 Kb

30 fragment was excised and purified.

The second round of PCR consisted of two separate reactions: reaction A, which used primers CRTESPHF and CRTERI, and reaction B, which used primers CRTESACR and CRTEFI. The sequences of primers CRTEFI and CRTERI were as follows.

5 CRTEFI 5'-GAGAGCGAGAGCCAGATCAAGAAGSGGCTG-
AAGGACATCC-3' (SEQ ID NO:174)
CRTERI 5'-GGATGTCCTTCAGCCSCTTCTTGATCTGGCT-
CTCGCTCTC-3' (SEQ ID NO:175)

10 The 20 nucleotides on the 3' ends of this pair of primers are located near the center of the crtE gene, 249 bases apart from each other and facing towards the start (CRTERI) and end (CRTEFI) of the gene. The 20 bp on the 5' ends of these primers are the reverse complement of the 3' end of the other primer in the pair. PCR of the two separate reactions was conducted as in the first round, with the exception that 0.05 ng of
15 first round product per μ L of reaction mix was used as template. Also, the thermocycler program used a 2 minute initial denaturation at 94°C; eight cycles of a 30 second denaturation at 94°C, a 45 second annealing at 56°C, and a 3 minute extension at 72°C, followed by eight cycles of a 30 second denaturation at 94°C, a 45 second annealing at 60°C, and a 3 minute extension at 72°C; followed by 16 cycles of a 30 second
20 denaturation at 94°C, a 45 second annealing at 64°C, and a 3 minute extension at 72°C; followed by a final extension at 72°C for 7 minutes. Both PCR products, about 590 and 650 bp in length, were separated on a 1% TAE-agarose gel, excised, and gel purified.

The third round of PCR used the same primers and reaction mixture as the first round of PCR with the exception that a mixture of 10 ng of each second round fragment
25 was used as template rather than genomic DNA (200 μ L reaction). The PCR program used was also the same as that used in the first round of PCR with the annealing time lengthened to 1.5 minutes. The 1.2 Kb third-round product was separated on a 1% TAE-agarose gel and purified. Three μ g of purified DNA was digested with the restriction enzymes SacI and SphI. The digested DNA was cleaned using a QIAquick PCR
30 Purification Kit and digested with the restriction enzyme StuI. StuI cut within the deleted

region and ensured that there was little or no remaining full-length product. The digestion mixture was again cleaned using a QIAquick PCR Purification Kit.

Three μg of the vector pL01 was digested with the restriction enzymes SphI and SacI. The enzymes were inactivated by heating to 65°C for 20 minutes, and the vector
5 was dephosphorylated using shrimp alkaline phosphatase (Roche). The dephosphorylated vector DNA was gel purified on a 1% TAE-agarose gel.

Sixty-six ng of digested vector DNA was ligated with 80 ng of the digested third-round PCR product at 16°C for 16 hours using T4 DNA ligase (Roche). One μL of ligation mix was electroporated into 40 μL of *E. coli* ElectroMAX™ DH5 α ™
10 electrocompetent cells (Life Technologies), which were then plated on LB media containing 50 $\mu\text{g}/\text{mL}$ kanamycin (LBK50). Plasmid DNA was isolated from cultures of individual colonies and digested with the restriction enzyme SacI and with a mixture of SphI and SacI to confirm correct vector structure.

One μL of plasmid DNA was used to transform electrocompetent cells of the
15 previously described *E. coli* strain S17-1. The electroporated cells were plated on LB media containing 25 $\mu\text{g}/\text{mL}$ of kanamycin, 25 $\mu\text{g}/\text{mL}$ of streptomycin, and 25 $\mu\text{g}/\text{mL}$ of spectinomycin (LBKSMST). Single colonies were used to start cultures for plasmid DNA isolation and used in conjugation. These colonies were also plated on LB media containing 5% sucrose and 25 $\mu\text{g}/\text{mL}$ of kanamycin to ensure that the *sacB* gene was still
20 functional. Only colonies which exhibited lethality on the sucrose media were used in conjugation. The presence of the correct insert size was confirmed by digestion of plasmid DNA with the restriction enzymes SacI and SphI.

Growing cultures of *R. sphaeroides* strain 35053 were sub-cultured, using 1/5 and 1/10 volumes of inoculum, in 5 mL Sistrom's media supplemented with 20% LB and
25 grown at 30°C for 12 hours. The S17-1 donor colonies were grown in LBKSMST media at 37°C for 12 hours. 1.5- 3.0 mL of each culture was pelleted, and the pellets were washed four times with LB media. Relative pellet size was estimated and about 2 volumes of 35053 cells were used to 1 volume of S17-1 cells. The cell mixture was pelleted, resuspended in 20 μL of LB media, spotted on an LB plate, and incubated at
30 30°C for 7- 15 hours. The cells were then scraped off the surface of the plate and

resuspended in 1.5 mL of Sistrom's salts. 200 μ L of resuspended cells were plated on each of seven plates of SisKTell media.

Colonies that grew on the plates after about 10 days, representing proposed single-crossover events, were streaked to new plates of the same media. Upon growth, single colonies were streaked out on LBK25 media. Purified colonies were patched to Sistrom's media supplemented with 1X LB, 15% sucrose, 0.5% DMSO (v/v), and 25 μ g/mL kanamycin (SisLBK15%SucDMSO). These were grown in an anaerobic chamber (Becton Dickinson, Sparks, MD) at 30°C for 5 days to check for lethality of the *sacB* gene in the proposed single-crossover events. Concurrently, the cultures were patched to SisLB media containing 15% sucrose and 0.5% DMSO (v/v) without kanamycin (SisLB15%SucDMSO). Several of the cultures exhibited both white and red colonies upon growth on this media. Whitish-gray colonies were purified from these cultures and tested by PCR to show that they contained the truncated *crtE* allele. These colonies were also screened using primers specific to the *R. sphaeroides* *ppsR* gene and the *E. coli* *dxs* gene as described above. Potential double crossovers were also streaked on LBK25 plates to confirm that they were now sensitive to kanamycin. The resulting *R. sphaeroides* mutant containing a *crtE* knockout was designated ATCC 35053/ Δ *crtE*.

Several discoveries were made using the *sacB* method to knockout nucleic acid sequenced within the *R. sphaeroides* genome. First, it was discovered that the cultures used in conjugations, particularly those of the recipient *R. sphaeroides* strain, should be in exponential growth. Second, it was discovered that when using the S17-1 strain as a vector donor, the use of telluride in the plating medium is unnecessary as this strain is a proline auxotroph and will not grow on Sistrom's media without LB supplementation. Third, it was discovered that potential single crossovers should be screened using two separate PCR reactions. The first reaction should use a primer within the gene of interest together with a primer homologous to upstream sequence. The second reaction should use a primer within the gene of interest together with a primer homologous to downstream sequence. One of these two reactions should produce a truncated fragment. Fourth, it was discovered that single crossovers that have been confirmed to have *sacB* lethality can be grown aerobically in Sistrom's media for 2 days and then plated on SisLB15%SucDMSO media. The volume plated varies depending on the rate of growth

of the strain, but is about one μL or less for strain 35053. This is then grown anaerobically for about 5 days. Fifth, it was discovered that the *sacB* gene may not completely kill cells with the gene, so there may be a background level of very small colonies. The desired double-crossover colonies, however, are typically larger. These colonies should be purified and screened by PCR to identify whether they contain the truncated or full-length allele. Sixth, it was discovered that using one primer homologous to sequence upstream of the knockout gene and one primer homologous to sequence downstream of the gene is useful in confirming the correct location of the insertion event in addition to determining the allele that is present.

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ATCC 35053/ Δ ppsR(strep)

R. sphaeroides cells lacking PPSR were made by inserting a spectinomycin/streptomycin resistance gene into the ppsR sequence as follows. To PCR amplify the ppsR gene from *R. sphaeroides* strain 17023, the following primers were designed based on published sequence (GenBank Accession Number L19596).

15

PPSRF2 5'-AGTCAGTACTAACTGGTGAAGACGCTGAAG-3' (SEQ ID NO:176)

PPSRR2 5'-GATCAGTACTGTGAACGAATACGATACGCA-3' (SEQ ID NO:177)

Each primer contained a *ScaI* restriction site. The ppsR gene was amplified using following reaction mix and PCR amplification program.

<u>Reaction Mix</u>		<u>Program</u>
25.	pfu 10X buffer	10 μL
	DMSO	5 μL
	dNTP mix (10 mM)	8 μL
	PPSRF2 (50 μM)	2 μL
	PPSRR2 (50 μM)	2 μL
30	Genomic DNA (50 ng/ μL)	2 μL
	pfu enzyme (2.5 U/ μL)	2 μL
	DI water	69 μL
	Total:	100 μL
		72°C 10 minutes
		4°C Until used further

35

The PCR product was separated on a 0.8% TAE agarose gel, and a band of about 1.8 Kb was cut and gel isolated using Qiagen Gel Isolation kit (Qiagen, Valencia, CA). The gel isolated DNA was digested with ScaI (New England BioLabs, Beverly, MA) for 5 hours. The digested DNA was column purified using Qiagen Gel Isolation kit. The cut
5 DNA was ligated into vector pSUP203 that was also digested with ScaI enzyme.

2.3 µg of pSUP203 plasmid DNA was digested for 4 hours at 37°C with ScaI restriction enzyme. The digested DNA was separated on a 1% TAE agarose gel. A 7.6 Kb fragment was excised and purified. The purified plasmid DNA was then dephosphorylated using calf intestinal phosphatase (New England Biolabs). 100 ng of
10 dephosphorylated plasmid DNA was ligated with 200 ng of the ScaI-digested PpsR DNA for 16 hours at 14°C using T4 DNA ligase (New England BioLabs). One µL of ligation mix was electroporated into 40 µL of *E. coli* ElectroMAX™ DH5α™ (Life Technologies, Gaithersburg, MD) electrocompetent cells, which were then recovered in 1 mL of SOC media for one hour at 37°C and plated on LB media containing 15 µg/mL tetracycline.
15 Plasmid DNA was isolated from 8 individual colonies using Qiagen spin Mini prep kit and digested with ScaI restriction enzyme to check insert size. Four of the colonies had a correct insert. 1.5 µg of the plasmid DNA obtained from confirmed colony was digested with XhoI restriction enzyme (New England BioLabs, Beverly, MA). This enzyme has a single restriction site in the open reading frame of ppsR gene. A linear DNA band of
20 about 8.4 Kb was gel isolated using a Qiagen Gel isolation kit. A spectinomycin/streptomycin resistance omega cassette was obtained by digesting plasmid pUI1638 (Obtained from Dr. Samuel Kaplan's laboratory) with XhoI enzyme. The digest was separated on a 0.8% TAE agarose gel, and a DNA band of about 2.1 Kb was gel isolated. This DNA which encoded for spectinomycin/streptomycin resistance gene was
25 ligated to pSUP203/PpsR, which was also restricted with XhoI enzyme. One µL of ligation mix was electroporated into 40 µL of *E. coli* ElectroMAX™ DH5α™ (Life Technologies, Gaithersburg, MD) electrocompetent cells, which were then recovered in 1 mL of SOC media for one hour at 37°C and plated on LB media with 15 µg/mL tetracycline, 25 µg/mL spectinomycin, and 25 µg/mL streptomycin. Plasmid DNA was
30 isolated from 10 individual colonies using Qiagen spin Mini prep kit and digested separately with ScaI and XhoI restriction enzyme to check insert size. Five of the

colonies had a correct insert. 100 ng of plasmid DNA from a confirmed colony was electroporated into electrocompetent cells of the *E. coli* strain SM10. This strain contains a chromosomal copy of the trans-acting elements that mobilize oriT-containing plasmids during conjugation with a second bacterial strain. It also carries a gene conferring resistance to the antibiotic kanamycin. The transformation reaction was recovered in 1 mL of SOC media for one hour and plated on LB media with 10 µg/mL tetracycline, 25 µg/mL kanamycin, 25 µg/mL of streptomycin, and 25 µg/mL spectinomycin.

The pSUP203/ppsR-SM-ST construct was conjugated from the *E. coli* SM10 host into *R. sphaeroides* strain 35053. The SM10 donor was grown in LB media with 25 µg/mL kanamycin, 25 µg/mL streptomycin, and 25 µg/mL spectinomycin at 37°C for 16 hours. A growing culture of *R. sphaeroides* strain 35053 was used to inoculate Sistrom's media in 1/5 to 1/10 dilutions. These cultures were grown for about 20 hours. Cells were pelleted for 1.5 mL of culture of both the SM10 pSUP203/PpsR-SM-ST and 35053 genotypes. Pellets were washed four times in Sistrom's media without vitamins and glucose. The pellets were each resuspended in 1.5 mL of Sistrom's media without vitamins and glucose. 200 µL of the SM10 pSUP203/PpsR-SM-ST cells were combined with 1.3 mL of the 35053 cells. This mixture was pelleted, the supernatant was removed, and the pellet was resuspended in 20 µL of LB media. The resuspended cells were spotted onto a LB plate that was then incubated at 30°C for 7 hours. The cells were then scrapped off the LB plate, resuspended in 1.5 mL of 1X Sistrom's media without vitamins and glucose, and plated (200 µL/plate) on Sistrom's media supplemented with 25 µg/mL spectinomycin, 25 µg/mL streptomycin, and 10 µg/mL of telluride. The telluride retards the growth of *E. coli* cells but is detoxified by *R. sphaeroides*. After 7-10 days, small black colonies were picked off the plates and streaked to fresh plates of the same media. After 6 days of growth, colonies were patched to LB plates containing 25 µg/mL spectinomycin and 25 µg/mL streptomycin (LBSMST25), and also to LB plates containing 0.75 µg/mL tetracycline. Desirable double-crossover events, in which the PpsR-SM-ST gene is retained in the genome and the vector DNA is lost, would have spectinomycin/streptomycin resistance but lack tetracycline resistance. Colonies resulting from undesirable single-crossover events would demonstrate resistance to all of these antibiotic markers.

Colonies that exhibited only spectinomycin/streptomycin resistance and displayed deep red color were confirmed for double-crossover by Southern hybridization. Southern hybridization was conducted on nineteen potential 35053/PpsR-SM-ST colonies in addition to 35053 and *R. sphaeroides* strain 17023. Sequence data for the photosynthetic operon of 17023 is available in Genbank and was used to determine restriction enzymes likely to have hybridization patterns that would distinguish mutants from non-mutants. Genomic DNA was isolated from each line using a Gentra Puregene DNA Isolation Kit (Gentra, Minneapolis, MN). 2 µg of genomic DNA was used in digests using the restriction enzymes NcoI, ApaI, and XmaI in separate reactions. The digests were separated on a 1% TAE agarose gel, and the DNA was transferred to nylon membrane (Roche Molecular Biochemicals, Indianapolis, IN). DIG-labeled molecular weight markers II and III (Roche) were also included on the gel/membrane. DIG-labeled probes of the PpsR locus were made using a PCR DIG Probe Synthesis Kit (Roche). After baking, membranes were prehybridized in EasyHyb Buffer (Roche) for at least 2 hours and hybridized overnight using 400 nL of a 0.5 DIG labeling per mL of hybridization solution. Detection was done using a Roche Wash and Block Buffer Set (Roche). Membranes were washed two times for 5-10 minutes at room temperature in 2X SSC/0.1% SDS and two times for 15-20 minutes at 68°C in 0.1X SSC/0.1% SDS. They were then covered with blocking buffer and placed on a shaker for an hour at room temperature. The blocking buffer was replaced with fresh blocking buffer containing 150 mU of AP conjugate per mL of buffer, and the membranes shaken at room temperature for an additional 30 minutes. Membranes were then washed twice for 15 minutes at room temperature with washing buffer, followed by a five minutes wash with detection buffer. The detection buffer was replaced with fresh detection buffer containing 20 µL of NBT/BCIP solution per mL of buffer. This was placed in the dark at room temperature with no shaking until sufficient color was developed.

In the NcoI digest, the lanes of colony 9 and 10 exhibited a band about 2 Kb larger than the 35053 control, which is the size difference expected from the insertion of the spectinomycin/streptomycin resistance cassette into the XhoI site. For the XmaI digest, 35053 exhibited a single band about 5.5 Kb, while colonies 9, 10, and 5 exhibited two bands whose summed size was about 2 Kb higher than that of 35053. Two bands were

observed in colony 9, 10, and 5 because a XmaI was introduced along with the spectinomycin/streptomycin resistance cassette. For ApaI digest, the control 35053 sample exhibited two bands since ppsR gene harbors an ApaI site. Each of these bands was about 2.3 Kb in size. Colony 9, 10, and 5 exhibited three bands, whose summed size was about 2 Kb higher band than that of 35053. An extra band was observed in colonies 9, 10, and 5 because an ApaI site was introduced along with the spectinomycin/streptomycin resistance cassette.

The resulting *R. sphaeroides* mutant containing the ppsR knockout was designated ATCC 35053/ Δ ppsR(strep).

10

ATCC 35053/ Δ ppsR

R. sphaeroides cells lacking ppsR were made using sacB selection as follows. A three-step PCR process was used to create a 255 bp in-frame deletion in the PpsR gene, so that there would be no residual antibiotic resistance gene in the genome. The PpsR gene from *R. sphaeroides* strain 35053 was amplified by PCR using primers designed to introduce an SacI restriction site at the beginning of the amplified fragment and a SphI restriction site at the end of the amplified fragment. The sequences of the primers were as follows.

20

PPSRSACF2 5'-GTCAAATGAGCTCCAAACTGGTGAAGA-
CGCTGAAGGACAT-3' (SEQ ID NO:178)

PPSRSPHR 5'-CAGTCGGGCATGCGTCCATTTCAGTTGAC-
ATACTTCTGTG-3' (SEQ ID NO:179)

25 The following PCR mix program was used to amplify the PpsR gene.

<u>Reaction Mix</u>		<u>Program</u>
pfu 10X buffer	10 μ L	94°C 2 minutes
DMSO	5 μ L	8 cycles of:
30 dNTP mix (10 mM)	3 μ L	94°C 30 seconds
PPSRSACF2 (100 μ M)	1 μ L	58°C 45 seconds
PPSRSPHR (100 μ M)	1 μ L	72°C 3 minutes
Genomic DNA (50 ng/ μ L)	2 μ L	25 cycles of:

	pfu enzyme (2.5 U/ μ L)	2 μ L	94°C	30 seconds
	DI water	76 μ L	64°C	45 seconds
			72°C	3 minutes
5	Total:	100 μ L	72°C	7 minutes
			4°C	Until used further

100 μ L of PCR product was separated on a 1% TAE agarose gel, and a fragment about 1.8 Kb was excised and purified using Qiagen Gel isolation kit.

The second round of PCR consisted of two separate reactions: reaction A, which used primers PPSRSACF2 and PPSRMIDR, and reaction B, which used primers PPSRSPHR and PPSRMIDF. The sequences of primers PPSRMIDF and PPSRMIDR were as follows.

PPSRMIDF 5'-CTCTTGCTCGGCGGCGTGCGGCTCTATCA-
 CGAGGGGGTGGGA-3' (SEQ ID NO:180)
 PPSRMIDR 5'-TCCACCCCCTCGTGATAGAGCCGCACGCC-
 GCCGAGCAAGAG-3' (SEQ ID NO:181)

The 20 nucleotides on the 3' ends of this pair of primers are located near the center of the ppsR gene, 255 bases apart from each other, and facing towards the start (PPSRMIDR) and end (PPSRMIDF) of the gene. The 20 bp on the 5' ends of these primers are the reverse complement of the 3' end of the other primer in the pair. The following reaction mix and program were used to conduct these PCR.

25	Reaction Mix A	Program
	pfu 10X buffer 10 μ L	94°C 2 minutes
	DMSO 5 μ L	8 cycles of:
	dNTP mix (10 mM) 3 μ L	94°C 30 seconds
	PPSRSACF2 (100 μ M) 1 μ L	58°C 45 seconds
30	PPSRMIDR (100 μ M) 1 μ L	72°C 3 minutes
	DNA from first round (10 ng/ μ L) 1 μ L	25 cycles of:
	pfu enzyme (2.5 U/ μ L) 2 μ L	94°C 30 seconds
		64°C 45 seconds
		72°C 3 minutes
35	DI water 77 μ L	72°C 7 minutes
	Total: 100 μ L	4°C Until further use

Reaction Mix B		Program
	pfu 10X buffer	10 μ L
	DMSO	5 μ L
5	dNTP mix (10 mM)	2 μ L
	PPSRSPHR (100 μ M)	1 μ L
	PPSRMIDF (100 μ M)	1 μ L
	DNA from first round (5ng/ μ L)	1 μ L
10	pfu enzyme (2.5 U/ μ L)	2 μ L
	DI water	78 μ L
Total:		100 μ L
		94°C 2 minutes
		8 cycles of:
		94°C 30 seconds
		58°C 45 seconds
		72°C 3 minutes
		25 cycles of:
		94°C 30 seconds
		64°C 45 seconds
		72°C 3 minutes
		72°C 7 minutes
		4°C Until further use

15 Both PCR products, about 800-700 bp in length, were separated on a 1% TAE agarose gel, excised, and gel purified using a Qiagen gel isolation kit.

The third round of PCR used primers PPSRSACF2 and PPSRSPHR but used both fragments derived in the second round of PCR as template. The PCR mixture used was the same as in the first round of PCR except that equal molar amounts of the round 2 fragments were used as template. The PCR program used was also the same as that used in the first round of PCR, with the annealing time lengthened to 1.5 minutes. The 1.5 Kb third-round product was separated on a 1% TAE agarose gel and purified using Qiagen gel isolation kit. The purified DNA was digested overnight at 37°C with the restriction enzymes SacI and SphI.

25 Three μ g of the vector pL01 was digested with the restriction enzymes SphI and SacI at 37°C for 16 hours. The enzymes were inactivated by heating to 65°C for 20 minutes. Dephosphorylation of the vector was achieved by adding 4.7 μ L of shrimp alkaline phosphatase 10X buffer (Roche) and 2 μ L of shrimp alkaline phosphatase to the inactivated digest. This mixture was heated at 37°C for 10 minutes and then 65°C for 15 minutes. The dephosphorylated vector DNA was then gel purified on a 1.0% TAE agarose gel.

98 ng of vector DNA was ligated with 210 ng of the digested third round PCR at 14°C for 14 hours using T4 DNA ligase (Roche). One μ L of ligation mix was electroporated into 40 μ L of *E. coli* ElectroMAX™ DH5 α ™ electrocompetent cells (Life Technologies), which were then recovered in 1 mL of SOC media for one hour and plated

on LB media with 25 µg/mL kanamycin (LBK25). Plasmid DNA was isolated from eight individual colonies. Plasmid DNA was checked for correct insert with a PCR screen using the PCR protocol from first round.

One µL of plasmid DNA was used to transform electrocompetent cells of *E. coli* strain S17-1. The electroporated cells were recovered in 1 mL of SOC media for one hour and plated on LB media with 25 µg/mL of kanamycin, 25 µg/mL of streptomycin, and 25 µg/mL of spectinomycin (LBKSMST). Single colonies were used to start cultures for plasmid DNA isolation and used in conjugation. These colonies were also plated on LB media containing 5% or 15% sucrose, and 25 µg/mL of kanamycin to ensure that the *sacB* gene was still functional. Only colonies that showed lethality on the sucrose media were used in conjugation. The presence of the correct insert size was confirmed by colony PCR.

Growing cultures of *R. sphaeroides* strain 35053 were subcultured, using 1/4 and 1/8 volumes of inoculum, in 5 mL Sistrom's media supplemented with 20% LB and grown at 30°C for 9 hours. The S17-1 donor colonies were grown in LBKSMST media at 37°C for 16 hours. 3.0 mL of 35053 and 0.5 mL of S17-1 donor cells were centrifuged and washed four times in Sistrom's media without glucose. Each cell pellet was resuspended into 20 µL LB, and the S17-1 donor suspension was mixed with 35053. The mixture was then spotted on LB, which was incubated at 30°C for 14-16 hours. The cells were then scraped off the surface of the plate and resuspended in 1.5 mL of Sistrom's salts. 200 µL of resuspended cells were plated on each of the seven Sistrom's media plates that were supplemented with 25 µg/mL of kanamycin.

Colonies that grew on the plates after about 10-14 days, representing proposed single crossover events, were streaked to new plates of the same media. Upon growth, single colonies were transferred to LBK25 media. These cultures were grown for 36 to 48 hours in Sistrom's media supplemented with 20% LB and no kanamycin at 30°C. 0.1 µL and 5 µL of this culture was plated on LB media that was supplemented with Sistrom's salts and 15% sucrose. The plates were placed in an anaerobic chamber (Becton Dickinson, Sparks, MD), and the chamber was placed in a 30°C incubator. After 4-5 days, several colonies showed up on the plates, indicating the occurrence of double-crossover events. Four colonies from each single-crossover strain were purified by

streaking on LB agar plates. Single colonies of double-crossover strains were screen by PCR for integration of truncated version of the ppsR gene into the chromosome. For screening, the following primers were used, which were located upstream and downstream of the PpsR gene. The use of upstream and downstream primer confirms
 5 both the locus of integration as well as truncation of PpsR gene.

PPSRUPF 5'-GAGCAGCACACTCTGGGAGC-3' (SEQ ID NO:182)

PPSRDNR 5'-CCACACAGGTAGGACACCCAC-3' (SEQ ID NO:183)

10 The following reaction mix and PCR program was used.

Reaction Mix		Program
Taq Mg+ 10X buffer	2.5 μ L	94°C 2 minutes
DMSO	1.25 μ L	29 cycles of:
15 dNTP mix (10 mM)	0.5 μ L	94°C 30 seconds
PPSRUPF (100 μ M)	0.125 μ L	61°C 45 seconds
PPSRDNR (100 μ M)	0.125 μ L	72°C 3 minutes
Cell boil mix	2 μ L	72°C 7 minutes
Taq enzyme (5 U/ μ L)	0.2 μ L	4°C Until further use
20 DI water	18.3 μ L	
Total:	25 μ L	

The cell boil mix was prepared by resuspending a single colony in 20-25 μ L of water. The suspension was heated at 95°C for 10 minutes in a PCR machine. The tube
 25 was given a quick spin to pellet the solids.

The colonies that exhibited the truncated version of the PpsR gene were further tested for kanamycin sensitivity by streaking them on LB plates that were supplemented with 25 μ g/mL of kanamycin. Also, these colonies were PCR screened for the kanamycin resistance gene.

30 The resulting *R. sphaeroides* mutant containing the ppsR knockout was designated ATCC 35053/ Δ ppsR.

ATCC 35053/ Δ ccoN

R. sphaeroides cells lacking ccoN were made using sacB selection as follows. A mutant of *R. sphaeroides* strain 2.4.1 having a 546 bp deletion in the ccoN gene (*R.*

sphaeroides 2.4.1/ Δ ccoN) was obtained from the laboratory of Samuel Kaplan at the University of Texas (Oh and Kaplan, *Biochemistry*, 38:2688-2696 (1999)). The mutated ccoN locus of this strain was amplified by PCR and cloned into pL01. This plasmid was transformed into *E. coli* strain S17-1. The S17-1 strain was conjugated with *R. sphaeroides* strain 35053, and colonies were identified in which the truncated locus had replaced the native ccoN gene.

The truncated ccoN gene from *R. sphaeroides* 2.4.1/ Δ ccoN was amplified by PCR using primers designed to introduce a SacI restriction site at the beginning of the amplified fragment and a SphI restriction site at the end of the amplified fragment. The sequences of the primers were as follows.

CCONSACF 5'-TCAGAGCTCGTGTGATCGAATGGGGCTTT-
GTTCTTGATG-3' (SEQ ID NO:184)

CCONSPHR 5'-GAAGCATGCAGGTGATCGACGTGCCACTC-
GTCCGAATAG-3' (SEQ ID NO:185)

The PCR reaction mix contained 0.2 μ M each primer, 1X Native Pfu reaction buffer, 0.2 mM each dNTP, 5% DMSO, and 10 units of Pfu DNA polymerase in a 200 μ L reaction. Three μ L of the glycerol stock was diluted in 20 μ L of 10 mM Tris and heated at 94°C for 10 minutes, after which 4 μ L was added to the PCR reaction. The PCR was conducted in a MJ Research PT100 and consisted of an initial denaturation at 94°C for 2 minutes; 32 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 66°C, and a 4 minute extension at 72°C, followed by a final extension at 72°C for 7 minutes. The PCR product was separated on a 1% TAE-agarose gel, and a 1.6 Kb fragment was excised and purified. Three μ g of purified PCR product was digested with SacI restriction enzyme and separated on a 1% TAE gel. A 1.4 Kb band was excised and purified. A SacI restriction site exists about 200 bp from the CCONSPHR end of the original PCR product.

Three µg of the vector pL01 was digested with the restriction enzyme SacI. The enzyme was inactivated by heating to 65°C for 20 minutes, and the digested vector was dephosphorylated using shrimp alkaline phosphatase. The dephosphorylated vector DNA was gel purified on a 1% TAE-agarose gel.

5 50 ng of digested vector DNA was ligated with 65 ng of the digested ccoN PCR product at 16°C for 16 hours using T4 DNA ligase (Roche). One µL of ligation mix was electroporated into 40 µL of *E. coli* Electromax™ DH5α™ electrocompetent cells, which were then plated on LBK media. Plasmid DNA was isolated from cultures of individual colonies and digested with the restriction enzyme SacI to confirm correct insert size:

10 The *E. coli* strain S17-1 contains a chromosomal copy of the trans-acting elements that mobilize oriT-containing plasmids during conjugation with a second bacterial strain. It also carries genes conferring resistance to the antibiotics streptomycin and spectinomycin. In addition, S17-1 is a proline auxotroph and will not grow on unsupplemented Sistrom's media. One µL of DNA of the truncated ccoN construct was
15 used to transform electrocompetent cells of *E. coli* strain S17-1. The electroporation was plated on LBKSMST. Single colonies were used to start cultures for plasmid DNA isolation and used in conjugation. These colonies were also plated on LB media containing 5% sucrose and 25 µg/mL of kanamycin to ensure that the sacB gene was still functional. Only colonies that exhibited lethality on the sucrose media were used in
20 conjugation. The presence of the correct insert size was confirmed by digestion of plasmid DNA with the restriction enzyme SacI.

 Growing cultures of *R. sphaeroides* strain 35053 were subcultured in Sistrom's media supplemented with 20% LB to ensure that they were in exponential growth. The S17-1 donor colonies were grown in LBKSMST media at 37°C overnight or subcultured
25 from growing colonies. 2-4 mL of each culture was centrifuged, and the pellets were washed four times in LB media. Relative pellet size was estimated, and about 2 volumes of 35053 cells were used to 1 volume of S17-1 cells. The cell mixture was then pelleted, resuspended in 20 µL of LB media, and spotted on an LB plate. This plate was incubated at 30°C for 7- 15 hours. The cells were then scraped off the surface of the plate and
30 resuspended in 1.2 mL of Sistrom's salts. 200 µL of resuspended cells were plated on each of six plates of Sistrom's media containing 25 µg/mL of kanamycin (SisK).

Colonies that grew on the plates after about 10 days, representing potential single-crossover events, were streaked to new plates of SisK media. Upon growth, single colonies were transferred to LBK media. Purified colonies were streaked to Sistrom's media supplemented with 1X LB, 15% sucrose, 0.5% DMSO (v/v), and 25 µg/mL kanamycin (SisLBK15%SucDMSO). These were grown in an anaerobic chamber (Becton Dickinson, Sparks, MD) at 30°C for 5 days to check for lethality of the *sacB* gene in the single-crossover events. The purified colonies were also screened in two separate PCR reactions. The first reaction used a primer within the gene of interest (CCONR) together with a primer homologous to upstream sequence (CCONUPF2), and the second reaction used a primer within the gene of interest (CCONSACF) together with a primer homologous to downstream sequence (CCONDNR2). Single-crossover events exhibited a truncated fragment in one of the two reactions, depending on whether the crossover occurred upstream or downstream of the deletion. The primer sequences were as follows.

15

CCONUPF2 5'-CTCACAACCTCCAACCGATG-3' (SEQ ID NO:186)

CCONR 5'-CGATGGTGACCACGAAGAAG-3' (SEQ ID NO:94)

CCONDNR2 5'-CGTAACGCTCGGTCTCGTC-3' (SEQ ID NO:129)

Single-crossover colonies were grown in Sistrom's media supplemented with 20% LB. After 2 days of growth, 0.1-1 µL of the cultures was plated on Sistrom's media supplemented with 1X LB, 0.5% DMSO (v/v), and 15% sucrose (SisLB15%SucDMSO). These cultures were grown anaerobically for about 5 days. The *sacB* gene did not always completely kill cells with the gene, so there was often a background level of very small colonies. The larger colonies, which represented double-crossover events, were purified on LB media and screened by PCR to identify whether they contained the truncated or full-length allele. The CCONUPF2 and CCONDNR2 primers were used in this PCR screen to ensure that the truncated gene also was inserted in the correct location in the genome. Potential double-crossovers were also streaked on LBK plates to confirm that they were now sensitive to kanamycin.

30

The resulting *R. sphaeroides* mutant containing the ccoN knockout was designated ATCC 35053/ Δ ccoN.

ATCC 35053/ Δ crtE/ Δ ccoN

5 *R. sphaeroides* cells lacking crtE and ccoN were made as follows. The wildtype ccoN allele of a crtE knockout mutant (ATCC 35053/ Δ crtE) was replaced with a truncated ccoN allele as described above. Double-crossover colonies having the truncated ccoN allele were then re-screened by PCR for the crtE and ccoN loci. These colonies were plated on LBK25 and screened by PCR to confirm the loss of the vector
10 from the genome. The resulting *R. sphaeroides* mutant containing the crtE knockout and ccoN knockout was designated ATCC 35053/ Δ crtE/ Δ ccoN.

ATCC 35053/ Δ crtE/ Δ ppsR/ Δ ccoN

15 *R. sphaeroides* cells lacking crtE, ppsR, and ccoN were made as follows. The wildtype ppsR allele of a crtE/ccoN knockout mutant (ATCC 35053/ Δ crtE/ Δ ccoN) was replaced with a truncated ppsR allele as described above with the following exceptions. After conjugation on an LB plate, the conjugated cells were plated on Sistrom's media containing 25 μ g/mL of kanamycin and 0.5% DMSO (SisKDMSO) rather than on SisK. After purification on SisKDMSO and LBKDMSO, single-crossovers were grown
20 aerobically in Sistrom's media supplemented with 1X LB and 0.5% DMSO. After 2 days of growth, the cultures were plated on Sistrom's media supplemented with 1X LB, 15% sucrose, and 0.5% DMSO, and grown anaerobically for 5 days. Potential double-crossover colonies were purified on LBDMSO and screened by PCR using the PPSRUPF and PPSRDNR primers. Colonies having the truncated ppsR allele were then rescreened
25 by PCR for the crtE, ppsR, and ccoN loci. These colonies were also plated on LBKDMSO and screened by PCR to confirm the loss of the vector from the genome. The resulting *R. sphaeroides* mutant containing the crtE knockout, ppsR knockout, and ccoN knockout was designated ATCC 35053/ Δ crtE/ Δ ppsR/ Δ ccoN.

Example 10 – Making recombinant microorganisms that
overexpress a particular sequence while a containing knock-out

Any construct developed for the overexpression of genes are transferred to any of the background genotypes developed by gene knockout techniques. For example, the
5 pMCS2tetP/Stdxs/Rsdds/EcUbiC or the pMCS2tetP/Stdxs/Rsdds/RsLytB construct is transferred into the *R. sphaeroides* ATCC 35053/ Δ crtE/ Δ ppsR/ Δ ccoN mutant cells to combine the productive effects of gene overexpression and engineering of gene regulation or carbon flow. The construct is transferred to the desired genotype by electroporation or conjugation. Conjugation of a plasmid into an *R. sphaeroides* strain follows the
10 procedure described for the isolation of single-crossover events except that, since the efficiency of plasmid transfer is much higher than that of chromosomal integration, a 0.1-1 μ L plating volume from the \sim 400 μ L conjugation recovery is ample to obtain transformed colonies. Single colony PCR is used to check the integrity of the construct in the new background, and evaluations of the productivity of the new strain are made.
15 Genes that are productive are integrated, in one or more copies, into appropriate regions of the chromosome of a productive strain along with or downstream of a highly-expressing promoter.

Example 11 - Three liter fermentations

20 Cultures of *R. sphaeroides* ATCC 35053 with various inserted genes or knockouts were grown in 5 mL culture tubes containing Sistrom's media with 4 g/L glucose. After 48 hours of growth at 30°C with 250 rpm shaking, the entire contents of the tube were used to inoculate a 300 mL baffled shake flask containing Sistrom's media with 4 g/L glucose. After incubation at 30°C for 48 hours, the entire contents of the flask were added
25 to 2.7 L of Sistrom's media containing 40 g/L glucose in a B. Braun Biotech International Model Biostat B fermenter.

The fermenter was maintained at 30°C, and the cascade was set to maintain the dissolved oxygen (DO) at 40%. The air inflow was maintained at 1 vvm, and the pH was maintained at 7.3 with an automatic feed of 2N NH₄OH. Foaming was controlled by
30 addition of Sigma Antifoam 289. Kanamycin to a concentration of 50 μ g/mL was added to fermentations with strains containing the broad host range vector pBBRIMCS2 either

with or without an inserted gene. At 24 to 30 hours, when the agitation increase to maintain a DO of 40% had leveled off, the agitation and DO were decoupled, and the agitation was fixed at 240 rpm. The air inflow was lowered to 0.3 vvm. Kanamycin to 50 µg/mL was again added to fermentations containing the expression vector.

- 5 The fermentation samples for coenzyme Q10 and spheroidenone analysis were removed at 69 to 75 hours into the fermentation.

Example 12 - Three-hundred milliliter fermentations

- Cultures of *R. sphaeroides* ATCC 35053 with various overexpressed genes or
10 knockouts were grown in 5 mL culture tubes containing Sistrom's media with 4 g/L glucose. After 48 hours of growth at 30°C with 250 rpm shaking, the entire contents of the tube were used to inoculate a 300 mL baffled shake flask containing Sistrom's media with 4 g/L glucose. After incubation at 30°C for 48 hours, 30 mL of the flask were added to 270 mL of Sistrom's media containing 40 g/L glucose in a 500 mL Infors AG-CH-
15 4103 fermenter.

- The fermenter was maintained at 30°C, and the cascade was set to maintain the dissolved oxygen (DO) at 40%. The air inflow was maintained at 1 vvm, and the pH was maintained at 7.3 with an automatic feed of 2N NH₄OH. Foaming was controlled by addition of Sigma Antifoam 289. Kanamycin to a concentration of 50 µg/mL was added
20 to fermentations with strains containing the broad host range vector pBBRIMCS2 either with or without an inserted gene. At 24 to 30 hours, when the agitation increase to maintain a DO of 40% had leveled off, the agitation and DO were decoupled, and the agitation was fixed at 400 rpm. The air inflow was lowered to 0.3 vvm. Kanamycin to 50 µg/mL was again added to fermentations containing the expression vector.

- 25 The fermentation samples for coenzyme Q10 and spheroidenone analysis were removed at 69 to 75 hours into the fermentation.

Example 13 - Analysis of Spheroidenone

- At various times during the fermentation, 15 mL of fermentation volume was
30 withdrawn. The volume of sample needed to obtain 5 mg of dry cell weight (DCW) was used for spheroidenone analysis. The sample was washed one time in water and

resuspended in an equal volume of water. The volume of sample calculated in step 1 was added to a 1.8 mL-microfuge tube and was centrifuged at 10,000 rpm for 3 minutes in an IEC MicroMax microfuge. The supernatant was removed, and the pellet was completely resuspended in 1.0 mL of Acetone:Methanol (7:2) and stored at room temperature away from light for 30 minutes. The sample was mixed once during this incubation. After incubation, the sample was centrifuged at 10,000 rpm for 3 minutes, and the extract (supernatant) collected. Samples were stored -20°C for analysis at a later time. The carotenoid extract was analyzed on a spectrophotometer scanning in the range of 350 nm to 800 nm, and the OD_{480} was recorded. The amount of carotenoid in mg/100 mL of culture was calculated using the following equation:

$$\text{Spheroidenone (mg) / 100 mL culture} = ((\text{OD}_{480} - (0.0816 * \text{OD}_{770})) * 0.484) / \text{Vol. of sample from step 1}$$

From mg of Spheroidenone/100 mL of culture, the amount of Spheroidenone/mg of dry cell weight (DCW) was calculated using the DCW number as the conversion factor. Care was taken to correct for any dilution factor required while the sample was scanned on the spectrophotometer.

Example 14 – Analyzing CoQ(10) levels produced via fermentation

100 mL of fermentation broth was removed once per day and placed in a tared 250 mL centrifuge bottle. The samples were centrifuged at 15,000 X g for 5 minutes, the supernatant was poured off, and the samples were resuspended in 50 mL cold water. The samples were centrifuged again at 15,000 X g for 5 minutes, and the supernatant was poured off. The wet weight of the biomass was determined, and the biomass was resuspended in 1.5 times its weight in water. The samples were stored covered with foil at -80°C before analysis.

Before analysis, the samples were warmed at 21°C for 15 minutes. 1.0 mL was withdrawn. Sodium dodecyl sulfate was added to a final concentration of 1.67 %. The samples were extracted with 14 mL of a hexane:ethanol (5:2) mixture. The samples were then evaporated to dryness and dissolved in 2 mL of a methanol:ethanol (9:2) mixture.

The samples were then analyzed on a Waters Nova-Pak C18 (3.9 x 150 mm: 4 μ m) column with a PDA detector set from 200-300 nm. Resolution was at 1.2 nm with a maximum absorbance at 275 nm. The run time was 15 minutes, and the injection volume was 20 μ L.

- 5 The dry weight of the samples were determined drying an aliquot at 105°C in an aluminum weighing pan for at least four hours.

Example 15 – Production of CoQ(10)

- 10 The following seven experiments measured the amount of CoQ(10) produced by the indicated microorganisms in a 3 liter scale fermentation.

In experiment 1, the following data were collected after 96 hours of fermentation:

Strain	Coenzyme Q10 (ppm) dry weight basis
ATCC 35053	2950
ATCC 35053/ Δ crtE	6508

These results demonstrated that the inactivation of crtE increased the production of CoQ(10).

- 15 In experiment 2, the following data were collected after 69 to 75 hours of fermentation:

Strain	Coenzyme Q10 (ppm) dry weight basis
ATCC 35053	1655
ATCC 35053/ Δ ppsR(strep)	3812

These results demonstrated that the inactivation of ppsR increased the production of CoQ(10).

20

In experiment 3, the following data were collected after 69 to 75 hours of fermentation:

Strain	Coenzyme Q10 (ppm) dry weight basis	Spheroidenone (ppm) dry weight basis
ATCC 35053	2951	1980
ATCC 35053/ Δ ccoN	3527	2959

These results demonstrated that the inactivation of ccoN increased the production of CoQ(10) and spheroidenone.

In experiment 4, the following data were collected after 69 to 75 hours of fermentation:

Strain	Coenzyme Q10 (ppm) dry weight basis
ATCC 35053/ Δ crtE	3255
ATCC 35053/ Δ crtE/ Δ ccoN isolate 8-7	7951

These results demonstrated that the inactivation of crtE and ccoN increased the production of CoQ(10) as compared to inactivating crtE only.

In experiment 5, the following data were collected after 69 to 75 hours of fermentation:

Strain	Coenzyme Q10 (ppm) dry weight basis
ATCC 35053/ Δ crtE	3545
ATCC 35053/ Δ crtE/ Δ ccoN isolate 111	4984
ATCC 35053/ Δ crtE/ Δ ppsR/ Δ ccoN	11,676

These results demonstrated that the inactivation of crtE and ccoN increased the production of CoQ(10) as compared to inactivating crtE only. In addition, these results demonstrated that the inactivation of crtE, ccoN, and ppsR increased the production of CoQ(10) as compared to inactivating only crtE and ccoN.

In experiment 6, the following data were collected after 69 to 75 hours of fermentation:

Strain	Coenzyme Q10 (ppm) dry weight basis
ATCC 35053/ Δ crtE	3833
ATCC 35053/ Δ crtE/pMCS2tetP/Stdxs	4928
ATCC 35053/ Δ crtE/pMCS2glnP/Stdxs	5508
ATCC 35053/ Δ crtE/pMCS2tetP/Stdds	4652

These results demonstrated that the inactivation of crtE together with the addition of Stdxs increased the production of CoQ(10) as compared to inactivating crtE only. In addition, these results demonstrated that the use of the gln promoter with Stdxs resulted in more production of CoQ(10) when compared to the use of the tet promoter with Stdxs. Further, these results demonstrated that the inactivation of crtE together with the addition of Stdds increased the production of CoQ(10) as compared to inactivating crtE only.

In experiment 7, the following data were collected after 69 to 75 hours of fermentation:

Strain	CoQ(10) (ppm) dry weight basis
ATCC 35053/pMCS2tetP	3909
ATCC 35053/pMCS2tetP/Stdxs/Rsdds	5387
ATCC 35053/pMCS2tetP/Stdxs/Rsdds/RsLytB	5962
ATCC 35053/pMCS2tetP/Stdxs/Rsdds/EcUbiC	6439

These results demonstrated that the addition of Stdxs and Rsdds increased the production of CoQ(10) as compared to adding vector only. In addition, these results demonstrated that the addition of either RsLytB or EcUbiC together with the addition of Stdxs and Rsdds increased the production of CoQ(10) as compared to adding only Stdxs and Rsdds.

The following four experiments measured the amount of CoQ(10) produced by the indicated microorganisms in a 300 mL scale fermentation.

In experiment 1, the following data were collected after 69 to 75 hours of fermentation:

Strain	CoQ(10) (ppm) dry weight basis
ATCC 35053/pMCS2tetP	5250
ATCC 35053/pMCS2tetP/Stdxs	5758
ATCC 35053/pMCS2tetP/Rsdds	6944
ATCC 35053/pMCS2tetP/Stdxs/Rsdds	6875
ATCC 35053/pMCS2tetP/Stdxs/Rsdds/EcUbiC	7808

- 5 These results demonstrated that the addition of either Stdxs or Rsdds increased the production of CoQ(10) as compared to adding vector only. In addition, these results demonstrated that the addition of Stdxs, Rsdds, and EcUbiC increased the production of CoQ(10) as compared to adding only Stdxs and Rsdds.

In experiment 2, the following data were collected after 69 to 75 hours of fermentation:

Strain	CoQ(10) (ppm) dry weight basis
ATCC 35053/pMCS2tetP	5483
ATCC 35053/pMCS2tetP/EcubiC	6360
ATCC 35053/pMCS2tetP/RsLytB	5976
ATCC 35053/pMCS2tetP/Stdxs/Rsdds/RsLytB	6751

10

These results demonstrated that the addition of either EcUbiC or RsLytB increased the production of CoQ(10) as compared to adding vector only. In addition, these results demonstrated that the addition of Stdxs, Rsdds, and RsLytB increased the production of CoQ(10) as compared to adding only RsLytB.

- 15 In experiment 3, the following data were collected after 69 to 75 hours of fermentation:

Strain	CoQ(10) (ppm) dry weight basis
ATCC 35053/pMCS2tetP	5072
ATCC 35053/pMCS2tetP/Stdxs/Rsdds/RsLytB	8050

These results demonstrated that the addition of Stdxs, Rsdds, and RsLytB increased the production of CoQ(10) as compared to adding vector only.

In experiment 4, the following data were collected after 69 to 75 hours of fermentation:

Strain	Coenzyme Q10 (ppm) dry weight basis
ATCC 35053/pMCS2tetP	4503
ATCC 35053/pMCS2tetP/Stdxs/Rsdds	8833

5

These results demonstrated that the addition of Stdxs and Rsdds increased the production of CoQ(10) as compared to adding vector only.

OTHER EMBODIMENTS

10

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

15

WHAT IS CLAIMED IS:

1. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:1 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (3626, 100), point B has coordinates (3626, 65), point C has coordinates (50, 65), and point D has coordinates (12, 100).
2. The isolated nucleic acid of claim 1, wherein said point B has coordinates (3626, 85).
3. The isolated nucleic acid of claim 1, wherein said point C has coordinates (100, 65).
4. The isolated nucleic acid of claim 1, wherein said point C has coordinates (50, 85).
5. The isolated nucleic acid of claim 1, wherein said point D has coordinates (15, 100).
6. The isolated nucleic acid of claim 1, wherein said nucleic acid sequence encodes a polypeptide.
7. The isolated nucleic acid of claim 6, wherein said polypeptide has DXS activity.
8. The isolated nucleic acid of claim 1, wherein said nucleic acid sequence is as set forth in SEQ ID NO:1.
9. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:2 over said length, wherein the

point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1926, 100), point B has coordinates (1926, 65), point C has coordinates (50, 65), and point D has coordinates (12, 100).

5

10. The isolated nucleic acid of claim 9, wherein said nucleic acid sequence encodes a polypeptide.

11. The isolated nucleic acid of claim 10, wherein said polypeptide has DXS activity.

10

12. An isolated nucleic acid comprising a nucleic acid sequence, wherein said nucleic acid sequence encodes a polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:3 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (641, 100), point B has coordinates (641, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100).

15

13. The isolated nucleic acid of claim 12, wherein said polypeptide has DXS activity.

20

14. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:37 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1990, 100), point B has coordinates (1990, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100).

25

15. The isolated nucleic acid of claim 14, wherein said point B has coordinates (1990, 85).

30

16. The isolated nucleic acid of claim 14, wherein said point C has coordinates (100,

- 55).
17. The isolated nucleic acid of claim 14, wherein said point C has coordinates (50, 85).
- 5 18. The isolated nucleic acid of claim 14, wherein said point D has coordinates (20, 100).
19. The isolated nucleic acid of claim 14, wherein said nucleic acid sequence encodes
10 a polypeptide.
20. The isolated nucleic acid of claim 19, wherein said polypeptide has DDS activity.
21. The isolated nucleic acid of claim 14, wherein said nucleic acid sequence is as set
15 forth in SEQ ID NO:37.
22. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:38 over said length, wherein the point defined by said length and said percent identity is within the area defined by points
20 A, B, C, and D of Figure 26, wherein point A has coordinates (1002, 100), point B has coordinates (1002, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100).
23. The isolated nucleic acid of claim 22, wherein said nucleic acid sequence encodes
25 a polypeptide.
24. The isolated nucleic acid of claim 23, wherein said polypeptide has DDS activity.
25. An isolated nucleic acid comprising a nucleic acid sequence, wherein said nucleic
30 acid sequence encodes a polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ

ID NO:39 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (333, 100), point B has coordinates (333, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100).

5

26. The isolated nucleic acid of claim 25, wherein said polypeptide has DDS activity.

27. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:40 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1833, 100), point B has coordinates (1833, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100).

15 28. The isolated nucleic acid of claim 27, wherein said point B has coordinates (1833, 85).

29. The isolated nucleic acid of claim 27, wherein said point C has coordinates (100, 65).

20

30. The isolated nucleic acid of claim 27, wherein said point C has coordinates (50, 85).

31. The isolated nucleic acid of claim 27, wherein said point D has coordinates (20, 25 100).

32. The isolated nucleic acid of claim 27, wherein said nucleic acid sequence encodes a polypeptide.

30 33. The isolated nucleic acid of claim 32, wherein said polypeptide has DDS activity.

34. The isolated nucleic acid of claim 27, wherein said nucleic acid sequence is as set forth in SEQ ID NO:40.

5 35. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:41 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1014, 100), point B has coordinates (1014, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100).

10

36. The isolated nucleic acid of claim 35, wherein said nucleic acid sequence encodes a polypeptide.

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37. The isolated nucleic acid of claim 36, wherein said polypeptide has DDS activity.

38. An isolated nucleic acid comprising a nucleic acid sequence, wherein said nucleic acid sequence encodes a polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:42 over said length, wherein the point defined by said length and said percent
20 identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (337, 100), point B has coordinates (337, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100).

25

39. The isolated nucleic acid of claim 38, wherein said polypeptide has DDS activity.

40. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:95 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (2017, 100), point B has
30 coordinates (2017, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100).

41. The isolated nucleic acid of claim 40, wherein said point B has coordinates (2017, 85).
- 5 42. The isolated nucleic acid of claim 40, wherein said point C has coordinates (100, 65).
43. The isolated nucleic acid of claim 40, wherein said point C has coordinates (50, 85).
- 10 44. The isolated nucleic acid of claim 40, wherein said point D has coordinates (20, 100).
45. The isolated nucleic acid of claim 40, wherein said nucleic acid sequence encodes
15 a polypeptide.
46. The isolated nucleic acid of claim 45, wherein said polypeptide has DXR activity.
47. The isolated nucleic acid of claim 40, wherein said nucleic acid sequence is as set
20 forth in SEQ ID NO:95.
48. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:96 over said length, wherein the point defined by said length and said percent identity is within the area defined by points
25 A, B, C, and D of Figure 26, wherein point A has coordinates (1161, 100), point B has coordinates (1161, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100).
49. The isolated nucleic acid of claim 48, wherein said nucleic acid sequence encodes
30 a polypeptide.

50. The isolated nucleic acid of claim 49, wherein said polypeptide has DXR activity.

51. An isolated nucleic acid comprising a nucleic acid sequence, wherein said nucleic acid sequence encodes a polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:97 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (386, 100), point B has coordinates (386, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100).

10

52. The isolated nucleic acid of claim 51, wherein said polypeptide has DXR activity.

53. An isolated nucleic acid comprising a nucleic acid sequence of at least 12 nucleotides, wherein said isolated nucleic acid hybridizes under hybridization conditions to the sense or antisense strand of a nucleic acid molecule, the sequence of said nucleic acid molecule being the sequence set forth in SEQ ID NO: 1, 2, 37, 38, 40, 41, 95, or 96.

15

54. The isolated nucleic acid of claim 53, wherein said nucleic acid sequence is at least 50 nucleotides.

20

55. The isolated nucleic acid of claim 53, wherein said nucleic acid sequence encodes a polypeptide.

56. The isolated nucleic acid of claim 53, wherein said polypeptide has DXS, DDS, or DXR activity.

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57. A substantially pure polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:3 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (641, 100), point B has coordinates (641, 65), point C has coordinates

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(25, 65), and point D has coordinates (5, 100).

58. The substantially pure polypeptide of claim 57, wherein said polypeptide has DXS activity.

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59. A substantially pure polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:39 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein
10 point A has coordinates (333, 100), point B has coordinates (333, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100).

60. The substantially pure polypeptide of claim 59, wherein said polypeptide has DDS activity.

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61. A substantially pure polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:42 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein
20 point A has coordinates (337, 100), point B has coordinates (337, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100).

62. The substantially pure polypeptide of claim 61, wherein said polypeptide has DDS activity.

25

63. A substantially pure polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:97 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein
30 point A has coordinates (386, 100), point B has coordinates (386, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100).

64. The substantially pure polypeptide of claim 63, wherein said polypeptide has DXR activity.
- 5 65. A host cell comprising an isolated nucleic acid of claim 1, 9, 12, 14, 22, 25, 27, 35, 38, 40, 48, 51, or 53.
66. The host cell of claim 65, wherein said host cell is prokaryotic.
- 10 67. The host cell of claim 65, wherein said host cell is selected from the group consisting of *Rhodobacter*, *Sphingomonas*, and *Escherichia* cells.
68. The host cell of claim 65, wherein said host cell comprises an exogenous nucleic acid that encodes a polypeptide having DDS, DXS, ODS, SDS, DXR, 4-
15 diphosphocytidyl-2C-methyl-D-erythritol synthase, 4-diphosphocytidyl-2C-methyl-D-erythritol kinase, or chorismate lyase activity.
69. The host cell of claim 65, wherein said host cell comprises an exogenous nucleic acid comprising an UbiC sequence or LytB sequence.
- 20 70. The host cell of claim 65, wherein said host cell comprises an exogenous nucleic acid comprising an UbiC sequence and LytB sequence.
71. The host cell of claim 65, wherein said host cell comprises non-functional crtE
25 sequence, ppsR sequence, or ccoN sequence.
72. The host cell of claim 65, wherein said host cell comprises non-functional crtE sequence, ppsR sequence, and ccoN sequence.
- 30 73. A host cell comprising an exogenous nucleic acid and a non-functional crtE sequence, ppsR sequence, or ccoN sequence, wherein said exogenous nucleic acid is

within a crtE, ppsR, or ccoN locus of said host cell.

74. A host cell comprising a genomic deletion, wherein said deletion comprises at least a portion of a crtE sequence, ppsR sequence, or ccoN sequence, and wherein said
5 host cell comprises a non-functional crtE sequence, ppsR sequence, or ccoN sequence.

75. A method for increasing production of CoQ(10) in a cell having endogenous DDS activity, said method comprising inserting a nucleic acid molecule comprising a nucleic acid sequence that encodes a polypeptide having DDS activity into said cell such that
10 production of CoQ(10) is increased.

76. The method of claim 75, wherein said nucleic acid molecule comprises an isolated nucleic acid of claim 14, 22, 25, 27, 35, 38, or 53.

15 77. The method of claim 75, wherein the production of CoQ(10) is increased at least about 5 percent as compared to a control cell lacking said inserted nucleic acid molecule.

78. The method of claim 75, wherein said cell is selected from the group consisting of *Rhodobacter* and *Sphingomonas* cells.
20

79. The method of claim 75, wherein said cell is a membraneous bacterium.

80. The method of claim 75, wherein said cell is a highly membraneous bacterium.

25 81. The method of claim 75, wherein said method further comprises inserting a second nucleic acid molecule comprising a nucleotide sequence that encodes a polypeptide having DXS activity into said cell.

82. The method of claim 81, wherein said second nucleic acid molecule comprises an
30 isolated nucleic acid of claim 1, 9, or 12.

83. A method for increasing production of CoQ(10) in a cell having endogenous DDS activity, said method comprising inserting a nucleic acid molecule comprising a nucleic acid sequence that encodes a polypeptide having DXS activity into said cell such that production of CoQ(10) is increased.

5

84. The method of claim 83, wherein the production of CoQ(10) is increased at least about 5 percent as compared to a control cell lacking said inserted nucleic acid molecule.

85. The method of claim 83, wherein said cell is selected from the group consisting of
10 *Rhodobacter* and *Sphingomonas* cells.

86. The method of claim 83, wherein said nucleic acid molecule comprises an isolated nucleic acid of claim 1, 9, or 12.

15 87. The method of claim 83, wherein said cell is a membraneous bacterium.

88. The method of claim 83, wherein said cell is a highly membraneous bacterium.

89. The method of claim 83, wherein said method further comprises inserting a
20 second nucleic acid molecule comprising a nucleotide sequence that encodes a polypeptide having DDS activity into said cell.

90. The method of claim 89, wherein said second nucleic acid molecule comprises an isolated nucleic acid of claim 14, 22, 25, 27, 35, 38, or 53.

25

91. A method for increasing production of CoQ(10) in a membraneous bacterium, said method comprising inserting a nucleic acid molecule comprising a nucleic acid sequence that encodes a polypeptide having DDS activity into said bacterium such that production of CoQ(10) is increased.

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92. A method for increasing production of CoQ(10) in a highly membraneous

bacterium, said method comprising inserting a nucleic acid molecule comprising a nucleic acid sequence that encodes a polypeptide having DDS activity into said highly membraneous bacterium such that production of CoQ(10) is increased.

- 5 93. A method for making an isoprenoid, said method comprising culturing a cell under conditions wherein said cell produces said isoprenoid, said cell comprising at least one exogenous nucleic acid that encodes at least one polypeptide, wherein said cell produces more of said isoprenoid than a comparable cell lacking said at least one exogenous nucleic acid.
- 10 94. The method of claim 93, wherein said cell is selected from the group consisting of *Rhodobacter* and *Sphingomonas* cells.
95. The method of claim 93, wherein said isoprenoid is CoQ(10).
- 15 96. The method of claim 93, wherein said at least one polypeptide has DDS, DXS, ODS, SDS, DXR, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase, 4-diphosphocytidyl-2C-methyl-D-erythritol kinase, or chorismate lyase activity.
- 20 97. The method of claim 93, wherein said at least one polypeptide is a UbiC polypeptide or a LytB polypeptide.
98. The method of claim 93, wherein said cell comprises a non-functional crtE sequence, ppsR sequence, or ccoN sequence.
- 25 99. The method of claim 93, wherein said cell comprises a non-functional crtE sequence, ppsR sequence, and ccoN sequence.
100. The method of claim 93, wherein said cell comprising a genomic deletion, wherein said deletion comprises at least a portion of a crtE sequence, ppsR sequence, or ccoN sequence, and wherein said cell comprises a non-functional crtE sequence, ppsR
- 30

sequence, or ccoN sequence.

101. A method for making an isoprenoid, said method comprising culturing a genetically modified cell under conditions wherein said cell produces said isoprenoid.

5

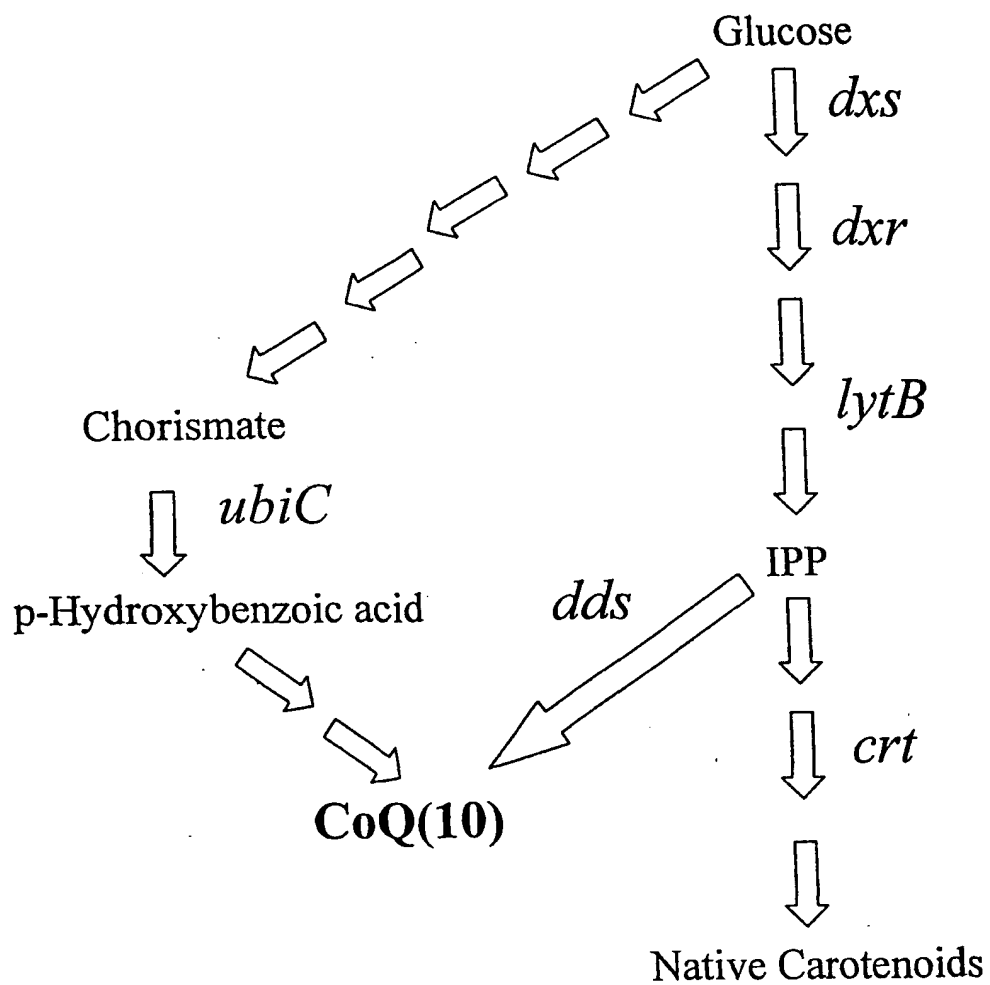
102. The method of claim 101, wherein said isoprenoid is CoQ(10).

103. The method of claim 101, wherein said cell comprises an exogenous nucleic acid.

10 104. The method of claim 101, wherein said cell comprises a genomic deletion.

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Figure 1



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Figure 2 (page 1 of 2)

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1  ctgcgccag accacgcata tcgacgacga ttcgatcacg aaaaacgtac
51  ggtccgcagc ccagcacgcc ggtttttcgc cggtcgggcc ggtgatcgag
101 gtgcgcgga agtgcgga gtgtgactga cctgtccaac agaccgttcg
151 acttgagact aacgttgcg taacaaagcc catggctgac ctaccaaga
201 cgccgctgct cgacacggtc gacacgccgc aggacctccg gaagctcgcc
251 cccgcccagc tgcgccagct ggccgacgag ctctgtgccg aaaccatcag
301 tgcggtgggc tccaccggcg ggcattctagg ctccggcctg ggcgtcgctg
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1851 acgaagtggc ggtgacgatc gaggaaggcg cgatcgggcg ccccggtgcg
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2051 gtgctgaagg cgctccgcta caacgaggcc gagctggccg acggggtgcg
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2151 gaaggcggtg gtggaggggc cgctgcggca cgancgggtt tcccaggctg

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Figure 2 (page 2 of 2)

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2201 agagcgatcc gcgccttgcg gcgcgcccc cccaccattc gctggcgcg
2251 atggtcccc tccccgttcc ggggaggatc tgggtcctgc cccaccttga
2301 atctccaaca tgcacatgcc atgtacatgc acatggctac gcagcttccc
2351 cagactcgct ccagccgcgt tgctgtgctg gtatcgcccg aggaaaaacg
2401 gcgcatttcc gccaatgcgg aagcggcgga catgacggtc agcgacttca
2451 tgcgcaccgc cgccgaacgc tataccgagc cgaccgacgc cgagatggcg
2501 ctgatgcgcy acctgctcgc ccagctcgaa caggccaatg cccgcacgga
2551 cgcggccttt gccagctcgc aagctgcgcy cgccgcccgc accgcgttcg
2601 acgaggaggc gtatcgcgcc gaggtccgcy aacagctgct gaccgatacc
2651 tcaatcgact gggatgcgct gtccactgcc ctttcgget gggcgcgcca
2701 gtgagcttct ggaccgatgc gctacgcgcy ctccagcagg tcgcgctgct
2751 ccagcacaag gtcgagcagg cgctgaccac cgccgaggaa gcccgccgcy
2801 attcaatcga gacgcgcgag cgggtgatcc ggcttgagac gctgatcgac
2851 atcgcgatga gacgccagcc cgcagcaccg cctacgcgcg ctgcgcttcc
2901 cgaaagtcca caaacgggca gctagcgccc gcttccccga gcgcgtacat
2951 cgcggtacgt gctgaaaatg accatccttc ccctcaccgc ccgcccccgcy
3001 gcgctcgcyg actggctgtt cgtcgtcgcc gcgatgatcg tcgcatgggt
3051 cgtggtcggg ggcattaccg ggctcaccga atcgggcctg tcgatcaccg
3101 aatggaagcc aatctccgcy atcgtgcccc cgctcaacga cgcgcagtgg
3151 caggccgagt tcgaccacta caagcagatc ggccagtatg agcagctcaa
3201 ccagggcatg acgctcggcg ggttcaagag catcttcttc tgggaatata
3251 tccaccgect gctcgccgcy ctgatcgga tgggtgttcg gctgccgctg
3301 ctgtgggttc ccgtccgcaa gcagatcccg cagggtatg gctggcggtc
3351 ggtcgcgctg ctcgcgctag gcgggctgca gggcgcgctc ggctggtgga
3401 tggatgaagtc ggggctcaac cacaccgca cctcggttag ccatttctgg
3451 ctggcgaccc acctgatgac cgcactgttc acgctgggcy gcatcgctg
3501 gacgatgctc gacctgcgcy cgcttgccgc caaccatgcc gagcgccctg
3551 cccgactgac cgggctcgcy gcgggcgtgc tgggtactgct ggcggtccag
3601 ctcttctacg gggcgctggt agcagg (SEQ ID NO:1)
```

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Figure 3

```

1  atggctgacc tacccaagac gccgctgctc gacacggctcg acacgccgca
51  ggacctccgg aagctcgccc ccgcccagct gcgccagctg gccgacgagc
101 ttcgtgccga aaccatcagt gcggtgggct ccaccggcgg gcatctaggg
151 tccggcctgg gcgtcgtcga actgacggtg gcgatccact atgtattcaa
201 cacccccgac gaccggctga tctgggacgt cgggcaccaa tgctatccgc
251 acaagatcct caccggtcgg cgcgatcgga tccgcacgat tcgtcagggg
301 ggaggcctct ccggcttcac caagcgcagc gagagcgagt atgatccgtt
351 cggtgcccg cactcgtcga cctcgatctc ggccgcactc ggctttgcga
401 tcgccaacaa gctcaacgag gcgcggggca aggcgatcgc ggtgatcggc
451 gacggcgcgga tgagcgcggg catggcctat gaggcgatga acaacgccga
501 ggccgccggc aaccggctgg tggatgacct caacgacaac gacatgtcga
551 tcgccccgcc ggtgggcggg ctttcggcct atcttgccgc cctcatttcc
601 tcgtccgaat atctcgccct gcgcgagctc gccaaagcgt tcaccgcaa
651 gctttcgcgc cgcctcaccg cggcagccgg caaggcggag gaattcgccc
701 gcggcatggc gaccggcggc acgctgttcg aggaacttgg cttctattat
751 gtcggcccga tcgacggcca caatctcgag catctgatcc cggtgctgga
801 gaatgtccgc gacagcgagc agggcccgat cctgatccat gtcgtgacca
851 agaagggcaa gggctatgcc ccggccgaag cggcggcgga caagtatcac
901 ggcgtccaga agttcgacgt gatcaccggg gcacaggcca aggcaccccc
951 gggcccgccc gcctatacca aggtgttcgc cgatgcgctg ctcgccgaag
1001 cggagcgtga tcggtcggtc tgccgcgatca ccgcggcgat gccctcgggc
1051 accgggctcg acaagttcca ggcgacgttc cccgatcgca cttcgcagct
1101 gggcattgcc gaacagcacg cgtcacctt cgcagcgggc cttgccgcgc
1151 aggggatgcg gccgttctgc gcgatctact cgaccttctt gcagcgcgcc
1201 tacgaccagg tcgtccacga cgtcgcgatc cagaacctgc cggtcgcctt
1251 cgcgatcgac cgcgcgggcc tggtcggtgc cgacggcgcg acccatgccg
1301 gcagcttcga cgtgacctat ctgccagcc tgcccaattt cgtggtgatg
1351 gcggccgcgg acgaggtcga gctcgtccac atgaccaca cggcggcgat
1401 gcacgacagc ggcccgatcg cgtgcgcta tccacgcggc aacggcgctg
1451 gactggcgct gcccaagggt ccggagcggc tggaaatcgg caagggtcgc
1501 gtggtccgag agggcaagaa ggtagcgatc ctgtcgctcg gcacgcgcct
1551 tgcggaagca ctaaaggccg ccgacacgct cgaggccaag ggcctctcga
1601 ccaccgtcgc cgacctgcgc ttcgccaaac cgctcgacga ggatctgatc
1651 cgccgcctgc tcaccacca cgaagtggcg gtgacgatcg aggaaggcgc
1701 gatcggcggc cccggtgcgc atgtgctgac gctcgccagc gataccggcc
1751 tgatcgacgc cggcctcaag ctgcgcacca tgccgctgcc ggacatattc
1801 caggaccagg acaagcccga gaagcagtat gacgaagcgg ggctgaacgc
1851 cgccaacatc gtcgacacgg tgctgaaggc gtcgcgtac aacgaggccg
1901 agctggccga cggggtgcgg gcgtaa (SEQ ID NO:2)

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Figure 4

```
1  madlpktp11 dtvdtppqdlr klapaqlrql adelraetis avgstggghlg
51  sglgvveltv aihyvfnfndp drliwdvghq cyphkiltgr rdrirtirgg
101 gglsgftkrs eseydpfgaa hsstsisaal gfaianklne apgkaiavig
151 dgamsagmay eamnnaeaag nrlvvilndn dmsiappvvg lsaylarlis
201 sseylglrel akrftrklr rltaaagkae efargmatgg tlfeelgfyy
251 vgpiddghnle hlipvlenvr dseqgpilih vvtkkkgkgya paeaaadkyh
301 gvqkfdivtg aqakappgpp aytkvfadal laeaerdasv caitaampsg
351 tglckfqatf pdrtfdvgia eqhavtfagg laaaggmrpfc aiystflqra
401 ydqvvhdvai qnlpvrfaid raglvgadga thagsfdvty laslpnfvm
451 aaadevelvh mthtaamhds gpialryprg ngvglalpkv perleigkgr
501 vvregkkvai lslgtrlaea lkaadt leak glsttvadlr fakpldedli
551 rrlittheva vtieegaigg pgahvltlas dtglidaglk lrtmrlpdif
601 qdqdkpekqy deaglnaani vdtvlkalry neaeladgvr a (SEQ ID
NO:3)
```

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STdxsdna	182	atg-----
CRdxsdna	1	atgctgcgtggtgctgtttctcacggccctgcggtcgccg
CJdxsdna	1	-----
PAdxsdna	1	atg-----
LEdxsdna	1	atg-----
MTdxsdna	1	-----
RSdxs1dna	1	atg-----
RSdxs2dna	1	atg-----
SPCCdxsdna	1	-----
ECdxsdna	1	atg-----
NMdxsdna	1	-----
HIdxsdna	1	atg-----
SSdxsdna	1	-----
HPdxsdna	1	-----
STdxsdna	185	-----gct-----
CRdxsdna	41	accgggctgccgct-----
CJdxsdna	1	-----at-----
PAdxsdna	4	-----cccaagacgctccatgagattccccgc--
LEdxsdna	4	-----gctttgtgtgcttatgcatttcctgggat
MTdxsdna	1	-----
RSdxs1dna	4	-----acc-----
RSdxs2dna	4	-----acc-----
SPCCdxsdna	1	-----
ECdxsdna	4	-----agtttt-----
NMdxsdna	1	-----
HIdxsdna	4	-----act-----
SSdxsdna	1	-----
HPdxsdna	1	-----gt-----
STdxsdna	188	-----
CRdxsdna	55	-----
CJdxsdna	3	-----
PAdxsdna	31	-----
LEdxsdna	33	tttgaacaggactggtgtggtttcagattcttctaaggca
MTdxsdna	1	-----
RSdxs1dna	7	-----
RSdxs2dna	7	-----
SPCCdxsdna	1	-----
ECdxsdna	10	-----
NMdxsdna	1	-----
HIdxsdna	7	-----
SSdxsdna	1	-----
HPdxsdna	3	-----

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STdxsdna	188	-----gacc---
CRdxsdna	55	-----ggcc---
CJdxsdna	3	-----ga-g---
PAdxsdna	31	-----gagc---
LEdxsdna	73	accacctttgttctctggatggattcatggaacagatc---
MTdxsdna	1	-----
RSdxs1dna	7	-----gaca---
RSdxs2dna	7	-----aatc---
SPCCdxsdna	1	-----
ECdxsdna	10	-----gata---
NMdxsdna	1	-----atg---
HIdxsdna	7	-----aacaata
SSdxsdna	1	-----
HPdxsdna	3	-----gatt---
STdxsdna	192	t-----ac-----cc-----
CRdxsdna	59	c-----cg-----cccgtgcgctgctcccg
CJdxsdna	6	t-----aa-----aa-----
PAdxsdna	35	g-----cc-----cc-----
LEdxsdna	110	t-----gcagtttttgttcc-----
MTdxsdna	1	-----
RSdxs1dna	11	g-----ac-----cc-----
RSdxs2dna	11	ccacccccgcgac-----cc-----
SPCCdxsdna	1	-----
ECdxsdna	14	t-----tg-----cc-----
NMdxsdna	4	a-----ac-----cc-----
HIdxsdna	14	t-----ga-----ac-----
SSdxsdna	1	-----
HPdxsdna	7	t-----tg-----ca-----
STdxsdna	197	-----
CRdxsdna	80	tcgcccgtggtgtgcgcagcgcagcgcacgcgtcagcg
CJdxsdna	11	-----
PAdxsdna	40	-----
LEdxsdna	125	-----
MTdxsdna	1	-----
RSdxs1dna	16	-----
RSdxs2dna	25	-----
SPCCdxsdna	1	-----
ECdxsdna	19	-----
NMdxsdna	9	-----
HIdxsdna	19	-----
SSdxsdna	1	-----
HPdxsdna	12	-----

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STdxsdna	197	-----
CRdxsdna	120	t c g c g c g g a g g c t t c g g t c a a t g c c c c g c g g g c g g c c c g
CJdxsdna	11	-----
PAdxsdna	40	-----
LEdxsdna	125	-----
MTdxsdna	1	-----
RSdxs1dna	16	-----
RSdxs2dna	25	-----
SPCCdxsdna	1	-----
ECdxsdna	19	-----
NMdxsdna	9	-----
HIdxsdna	19	-----
SSdxsdna	1	-----
HPdxsdna	12	-----
STdxsdna	197	-----
CRdxsdna	160	g c c g g t a g c t a c t c g g g c g a g t g g g a t a a g c t t t c a g t g g
CJdxsdna	11	-----
PAdxsdna	40	-----
LEdxsdna	125	-----
MTdxsdna	1	-----
RSdxs1dna	16	-----
RSdxs2dna	25	-----
SPCCdxsdna	1	-----
ECdxsdna	19	-----
NMdxsdna	9	-----
HIdxsdna	19	-----
SSdxsdna	1	-----
HPdxsdna	12	-----
STdxsdna	197	-----aag--acg
CRdxsdna	200	a g g a g a t t g a t g a g t g g c g c g a t g t g g g c c c g a a g --a c g
CJdxsdna	11	-----aat--ttg
PAdxsdna	40	-----gcc--acg
LEdxsdna	125	-----aac--aca
MTdxsdna	1	-----
RSdxs1dna	16	-----tgc--acg
RSdxs2dna	25	-----gaa--acc
SPCCdxsdna	1	-----atg
ECdxsdna	19	-----aaa--tac
NMdxsdna	9	-----aag---c
HIdxsdna	19	-----aat--tat
SSdxsdna	1	-----gtg
HPdxsdna	12	-----aaataaaa

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STdxsdna	203	ccgctgctc-----gacacggtcga-----ca
CRdxsdna	238	cccctgctg-----gacactgtcaa-----tt
CJdxsdna	17	cccatactc-----aa-----
PAdxsdna	46	cccctgctc-----gaccgcgcctc-----tt
LEdxsdna	131	agcttactcatgaggtcaagaaaaggtcacgtgtggttca
MTdxsdna	1	---atgctg-----caacagatccg-----cg
RSdxs1dna	22	ccgacgctc-----gac-cgggtga-----cg
RSdxs2dna	31	ccgcttttg-----gatcgcgctctg-----ct
SPCCdxsdna	4	catctcagc-----gaaa---ttac-----cc
ECdxsdna	25	ccgaccctg-----gcactgggtcga-----ct
NMdxsdna	13	cccctactc-----gacctgattga-----ca
HIdxsdna	25	cctctttta-----tctttaattaa-----tt
SSdxsdna	4	acgattctg-----gagaacatccg-----gc
HPdxsdna	20	cttttgatt-----taaaccctaac-----ga
STdxsdna	225	cgcc-gcaggacc---tccgga-----ag
CRdxsdna	260	accc-ggtgcacc---tgaaga-----ac
CJdxsdna	28	-----gaagagt---tagaaa-----ag
PAdxsdna	68	cgcc-ggccgaac---tgcgcc-----gg
LEdxsdna	171	ggct-tccttatcagaatctggagaatactacacacagag
MTdxsdna	20	ggcc-cgctgatc---tgcagc-----ac
RSdxs1dna	43	ctcccgggtggaca---taaagg-----gc
RSdxs2dna	53	gccc-ggccgaca---tgaagg-----cg
SPCCdxsdna	23	atcc-caaccagc---tccacg-----gg
ECdxsdna	47	ccac-ccaggagt---tacgac-----tg
NMdxsdna	35	gccc-gcaagatt---tgcgcc-----gt
HIdxsdna	47	ctcc-agaagatt---tgcgtc-----tt
SSdxsdna	26	aacc-acgcgacc---tgaagg-----cg
HPdxsdna	42	tatt-gcagg-----cttgg-----ag
STdxsdna	245	ctcgcccccgcccagctgcgccag-----
CRdxsdna	280	ttcaacaatgagcagctgaagcag-----
CJdxsdna	43	ctaagttaaagaattagaaaat-----
PAdxsdna	88	ctgggcgaggcgacctggaaacc-----
LEdxsdna	210	accgccaacgcctattttggacactgtgaactatccatt
MTdxsdna	40	ctttcccaggcgagcttcgggag-----
RSdxs1dna	64	ctcacggaccgtgagttgcgctcg-----
RSdxs2dna	73	ctgagtgacgccgaactggagcgg-----
SPCCdxsdna	43	ttgtcggttgctcagcttgagcaa-----
ECdxsdna	67	ttgccgaaagagagtttaccgaaa-----
NMdxsdna	55	ctggacaaaaaacagctgccgcgc-----
HIdxsdna	67	ttaaataaagatcagctaccacaa-----
SSdxsdna	46	ctgcccaggagcagctgcacgaa-----
HPdxsdna	58	tt-----ggtgtgtcaa-----

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STdxsdna	269	-----ctgg
CRdxsdna	304	-----ctct
CJdxsdna	67	-----ttag
PAdxsdna	112	-----ctgg
LEdxsdna	250	catatgaaaaatctgtctctgaaggaaacttaaacaactag
MTdxsdna	64	-----ctgg
RSdxs1dna	88	-----ctgg
RSdxs2dna	97	-----ctgg
SPCCdxsdna	67	-----attg
ECdxsdna	91	-----ctct
NMdxsdna	79	-----cttg
HIdxsdna	91	-----ctct
SSdxsdna	70	-----ctgt
HPdxsdna	70	-----acg-
STdxsdna	273	ccgacgagcttcgtgccgaaacca-tcagtg--cgggtggg
CRdxsdna	308	gcaaggagctgcgcagtgacatcg-tgcaca--ccgtctc
CJdxsdna	71	cagcatctatgcgtgaaaaaatca-tacaag--ttgtgag
PAdxsdna	116	ccgacgagct--gcgccagtacct-gctgtataccgtcgg
LEdxsdna	290	cagatgaactaaggctcagatacaa-ttttca--atgtatc
MTdxsdna	68	ccgccgagatccgtgagttcctga-tccaca--aggttgc
RSdxs1dna	92	ccgacgagctgcgggccgaaacga-tctcgg--ccgtgtc
RSdxs2dna	101	ccgacgaagtgcgttccgaggtga-tttcgg--tcgttgc
SPCCdxsdna	71	gccaccagattcgtgagaagcacc-tgcaga--cggttgc
ECdxsdna	95	gcgacgaactgcgccgctatttac-tgcaga--gcgtgag
NMdxsdna	83	ccggcgagttgcgcacctttctgc-tggaat--ctgtcgg
HIdxsdna	95	gtcaagaattacgtgcttatcttt-tagaat--ctgttag
SSdxsdna	74	ccgaggaga-tcaggcagttcctggtgcacg--cggtcac
HPdxsdna	73	-ctacg-gaatcgt-----attd-tagaag--tggtgag
STdxsdna	310	ctccaccggcgggcatctaggctccggcctgggctgctc
CRdxsdna	345	tcgcaccggtggacaccttagcagcagcctgggctggtg
CJdxsdna	108	taaaaatggtgggcatttaagttcaaatttggtgctgta
PAdxsdna	153	ccagaccggcgggtcatttcggcgccggcctcggcgtggtc
LEdxsdna	327	aaagactgggggtcaccttgggtcaagtcttggtgttgtt
MTdxsdna	105	cgccacgggggggcatctggggccgaacctgggagtggtg
RSdxs1dna	129	ggtgacggggcgggcatctggggcgaggcctcggcgtggtg
RSdxs2dna	138	cgagacgggaggacatctgggggtcctcgctgggggtggtt
SPCCdxsdna	108	agcgaccggtgggcacctcgggcgggcttgggctggtg
ECdxsdna	132	ccgttcagcgggcacttcgcctcgggctgggcaagggtc
NMdxsdna	120	gcagaccggcgggcatttcgccagcaatttgggcgcggtc
HIdxsdna	132	tcaaactagcggacatttagcgtcaggtttaggcactgta
SSdxsdna	111	cagaaccggcgggtcatctgggacccaacctgggggtggtg
HPdxsdna	102	cgctaattggggggcatttaagctcttctttaggggctgtg

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STdxsdna	350	gaactgacggtggcgatccactatgtattcaacacccccg
CRdxsdna	385	gagctgacggtggctatgcactatgtattcaacacccccg
CJdxsdna	148	gaacttagtatagcaatgcatttggtttttgatgcaaaaa
PAdxsdna	193	gagctgaccattgccctgcactacgtcttcgacactccgg
LEdxsdna	367	gagctgactgttgctcttcattatgtcttcaatgcaccgc
MTdxsdna	145	gaactcaccttggcgctgcaccgggtattcgactcgccgc
RSdxs1dna	169	gagttgacggttgcgctgcacgcatcttcgatgcgcccc
RSdxs2dna	178	gagctgactgtcgcgctgcacgcatcttcgatgcgcccc
SPCCdxsdna	148	gaattgaccctagcgctttaccaaagcgtcgatctcgatc
ECdxsdna	172	gaactgaccgtggcgctgcactatgtctacaacacccccg
NMdxsdna	160	gagctgacggttgcgctgcactacgtttacaacacgcccc
HIdxsdna	172	gagctaaccgttgcgctgcattatgtatataagacgccat
SSdxsdna	151	gagctgaccatcgccctgcaccgggtcttcgagtcgcccc
HPdxsdna	142	gagctgattgtgggcatgcacgcttatttgattgcaaaa
STdxsdna	390	acgaccggctgatctgggacgtcgggcaccaatgctatcc
CRdxsdna	425	aggacaagattatgtgggacgtgggcccaccaggcgtatgg
CJdxsdna	188	aagatccttttatttttgatgtgtcgcatcagtccttatac
PAdxsdna	233	acgaccgcctggtctgggacgtcggcccaccaggcctatcc
LEdxsdna	407	aagataggattctctgggatgttggtcatcagtccttacc
MTdxsdna	185	acgatccgatcatcttcgacacccggtcaccaggcctacgt
RSdxs1dna	209	gcgacaagatcatctgggacgtgggcccaccagtgtatccc
RSdxs2dna	218	ccgacaagctcgtctgggacgtgggcccaccagtgtatccc
SPCCdxsdna	188	gcgacaaagtgggttgggacgttggcccaccaagcctatcc
ECdxsdna	212	ttgaccaattgatttgggatgtggggcatcaggccttacc
NMdxsdna	200	aagacaagctggtgtgggatgtcggacaccaaagctatcc
HIdxsdna	212	ttgatcagtttaatttgggatgtgggacatcaagccttacc
SSdxsdna	191	tcgaccgcacccctgtgggacacccggccaccagagctacgt
HPdxsdna	182	aaaaccctttcatttttgacacttcgcaccaagcctacgc
STdxsdna	430	gcacaagatcctcaccgggtcggcgcgatcgga---tccgc
CRdxsdna	465	ccacaagatcctgactggcgtcgcaagggt---tggcc
CJdxsdna	228	acacaagcttttaagcggaaaagaagaatat---ttgat
PAdxsdna	273	gcacaagatcctcaccgagcgccgcgagctga---tgggc
LEdxsdna	447	tcacaaaatcttgactggtagaagggaaga---tgtcg
MTdxsdna	225	ccacaagatgttgaccggacgcagccaggact---tcgca
RSdxs1dna	249	ccacaagatcctgaccggcgcgcgacccgca---tccgc
RSdxs2dna	258	ccacaagatcctcaccggccggcgcgagcaga---tgcgc
SPCCdxsdna	228	ccacaagctgctgacag---ggcgctatcacacttccat
ECdxsdna	252	gcataaaattttgaccggacgcgcgacaaaa---tcggc
NMdxsdna	240	gcacaaaattcttaccggacgtaaaaaccaga---tgcac
HIdxsdna	252	acataaaatcctaaccgggtcgccgagagcaaa---tgtcc
SSdxsdna	231	acacaagctgctgacgggacgtcagga---ct---tctcc
HPdxsdna	222	ccacaagcttttaaccggcgctttgaaagct---ttagc

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STdxsdna	467	acgattcgtcaggggtggaggcctctccggcttcaccaag-
CRdxsdna	502	acgattcgcagaccaacggcctttcgggcttcacgaag-
CJdxsdna	265	actttaagacaaatcaatggtttaagtggttatacaaaa-
PAdxsdna	310	accctgcgccagaagaacggcctggcgcccttcccgcg-
LEdxsdna	484	acattaaggcagacagatgggtcttgcaggatttactaag-
MTdxsdna	262	accctgcgtaagaagggcggttgcggggtatccgtct-
RSdxs1dna	286	accctgcggcagggcggggtctctcgggcttcaccaag-
RSdxs2dna	295	accctgcgccagaagggcgccctctcgggcttcaccaag-
SPCCdxsdna	265	accttgcggcaaaaaggatggcattgcgggctaccggaag-
ECdxsdna	289	accatccgtcagaaaaggcggtctgcacccgttcccggtg-
NMdxsdna	277	accatgcgccaatatggcggtttggcggttttccgaaa-
HIdxsdna	289	acaattcgccaaaaaagacgggtat-tcatccttttcttgg
SSdxsdna	265	aagctgcgcggcaagggcgccgtgtccggctaccctcg-
HPdxsdna	259	actttaaggcaattcaagggtttgagcggtttactaaa-
STdxsdna	506	cgcagcgagagcgagtatgatccgttcgggtgccgcgc-ac
CRdxsdna	541	cgcgacgagagcgagtacgaccctttcggcgctggcc-ac
CJdxsdna	304	cctagcgaggagattat-----tttgtagcagggc-at
PAdxsdna	349	cgcgacgagagcgagtacgacaccttcggcgctcgcc-ac
LEdxsdna	523	cgatcggagagtgaatatgattgctttggcaccggcc-ac
MTdxsdna	301	cgtgccgagagcgagcacga-ctgggtggagtcgagccac
RSdxs1dna	325	cgctccgagagcccctatgactgtttcggcgcgggcc-at
RSdxs2dna	334	cgctcggaatccgcctacgaccggttcggcgcggtc-at
SPCCdxsdna	304	cgcacggaaccgcttcgatcatttcgggtgccggtc-ac
ECdxsdna	328	cgcggcgaaagcgaatatgacgtattaagcgtcgggc-at
NMdxsdna	316	cgttgcgagtcgagtagcagcggttcggcggtggggc-at
HIdxsdna	328	cgtgaagaaagtgaatttgatgtattaagtgttggtc-ac
SSdxsdna	304	cgcgaggagtcgagcacgacgtcatcgagaacagcc-ac
HPdxsdna	298	cccagcgagagcgcatacgattatttcacgcggggc-at
STdxsdna	545	tcgtcgacctcgatctcggccgcact--cggctttgcgat
CRdxsdna	580	agctccacctcgatttcggcggtct--gggtatggcggt
CJdxsdna	337	tctagtacctctatttctttggcagt--agggtcttgtaa
PAdxsdna	388	tccagcacctccatcagcgccgcct--gggcatggccat
LEdxsdna	562	agttccaccaccatctcagcaggcct--agggatggctgt
MTdxsdna	340	gccagcgcgcgctgtcgtacgcgga--cgggttgccaa
RSdxs1dna	364	tcctcgacctcgatctcggccgcggt--gggctttgccgc
RSdxs2dna	373	tcctcgacctcgatctcggccgcgct--cggctttgccat
SPCCdxsdna	343	gcttccaccagtatttctgtgtgcct--cggtatggctct
ECdxsdna	367	tcatcaacctccatcagtgccggaat--tggtattgcggt
NMdxsdna	355	tcctccacctccatcggcgcggtt--gggcatggcggc
HIdxsdna	367	tcctctacgtctattagtgcgggatt--aggcattgccgt
SSdxsdna	343	gcctccac--cgccctcgggtgggcccgcgactcgccaa
HPdxsdna	337	agttccacttcgggtgt-----ctat--aggcggtggggt

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STdxsdna	583	cgc-c-----aacaagctc-----aacgag-gc--
CRdxsdna	618	ggg-c-----cgcgacgtt-----aagggc-aa--
CJdxsdna	375	ggc-t-----attgcttta-----aagggg-ga--
PAdxsdna	426	cgc-c-----gccgcctg-----caaggc-aa--
LEdxsdna	600	tgg-t-----agagatcta-----aaagg-a-g--
MTdxsdna	378	ggc-g-----ttcgagttg-----accg-g-ac--
RSdxs1dna	402	ggc-a-----cgcgagatg-----ggcggc-ga--
RSdxs2dna	411	ggg-t-----cgcgagctg-----ggccag-cc--
SPCCdxsdna	381	agcac-----gggatgcc-----agggcg-aa--
ECdxsdna	405	tgc-tgccgaaaaagaaggca-----aa--aa-tc--
NMdxsdna	393	ggc-g-----gacaaacag-----ttgggcagc--
HIdxsdna	405	tgc-c-----gcag-----aacgag-aaaa
SSdxsdna	381	ggc-c-----cgccgggtg-----cagggg-ga--
HPdxsdna	369	ggc-t-----a--aagctttttgtttgaaacaa-gc--
STdxsdna	604	-gccgg--gcaaggc----gatcgcggtgatcggcgacgg
CRdxsdna	639	-gaaga--acagtgt----gatcgctgtcatcggcgacgg
CJdxsdna	396	-aaagc--gtattcc----tgttgctttgattggagatgg
PAdxsdna	447	-ggagc--gtaagtc----ggtggccgtgatcggcgacgg
LEdxsdna	621	-aaaca--acaatgt----tattgccgtaataggtgatgg
MTdxsdna	398	-accgc--aaccggcatgtggtcgcggtggtcggcgacgg
RSdxs1dna	423	-cacgg--gcgacgc----ggtggcggtgatcggcgacgg
RSdxs2dna	432	-cgtgg--gcgacac----gatcgccgtgatcggcgacgg
SPCCdxsdna	403	-gacta--ccgatgt----g-tcgctgtgattgggtgatgg
ECdxsdna	431	-gcc----gca---c----cgtctgtgtcattggcgatgg
NMdxsdna	415	-gaccg--ccgcagc----g-tcgccatcatcggcgacgg
HIdxsdna	423	tgcaggtagaaaaaac----agtatgcgtaatcgggtgatgg
SSdxsdna	402	-gaagg--gccatgt----cgtcgccgtcatcggcgacg
HPdxsdna	396	-gctag--gcatgcc----catagctttattaggcgatgg
STdxsdna	637	cgcgatgagcgcgggcatggcctatgaggcgatgaacaac
CRdxsdna	672	cgccatcacgggggtatggcctatgaggccatgaaccat
CJdxsdna	429	tgctttaagtgcgggtatggcctatgaggctttaaatgaa
PAdxsdna	480	tgcgctgaccgccggcatggccttcgaggcactcaaccac
LEdxsdna	654	tgccatgacagcaggtcaagcttatgaagccatgaataat
MTdxsdna	435	tgcgctcaccggcggtatgtgctgggaggcgctgaacaat
RSdxs1dna	456	ctcgatgtcgccggcatggccttcgaggcgctgaaccac
RSdxs2dna	465	ctccatcacggcgggcatggcctacgaggcactgaaccac
SPCCdxsdna	435	atcgctcaccgggtggcatggccttggaagccatcaaccac
ECdxsdna	459	cgcgattaccgcaggcatggcggttggaagcgatgaatcac
NMdxsdna	447	cgcgatgacggcggtcaggcggttggaagccttgaactgc
HIdxsdna	459	cgcaattactgcgggaatggcatttgaggcattaaatcac
SSdxsdna	435	ggcgctgaccggcggtatggcctgggaggccctgaacaac
HPdxsdna	429	gagcattagtgcagggattttttatgaagccttaaacga-

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STdxsdna	677	gccgaggcc--gccgg--caa--c-cggc-----t--gg
CRdxsdna	712	gcgggcttc--ctgga--caa--g-aaca-----t--ga
CJdxsdna	469	ttgggtgat--tctaa--att--t-cctt-----g--cg
PAdxsdna	520	gcctcggaa--gtcga--cgc--c-gaca-----t--gc
LEdxsdna	694	gc--tgggt--acctg--gac--t-ctgaca----t--ga
MTdxsdna	475	atc--gcc--gcac--ccg--c-cggc-----c--gg
RSdxs1dna	496	ggcgggcac--ctgaa--gaa--c-cggg-----t--ga
RSdxs2dna	505	gc--gggcc--atctgaacaa--g-cgcc-----t--gt
SPCCdxsdna	475	gctggtcacttgccca--aaa--cacggc-----t--gt
ECdxsdna	499	-----gcg--ggcga--tat--c-cgtcctgatat--gc
NMdxsdna	487	gc--gggcg--atatg--gat--g-tgga-----tttgc
HIdxsdna	499	gcgggggc---attg--cat--a-caga-----tatgt
SSdxsdna	475	atcgcggcc--gccaa--gga--c-cagc-----c--gc
HPdxsdna	468	-actgggcg--atagg--aaatac-ccca-----t--ga
STdxsdna	702	tggtgatcct---c---aacgacaac-gaca---tgtcga
CRdxsdna	737	ttgtgattct---g---aacgacaac-cagcaggtgtcgc
CJdxsdna	494	taatactttt---a---aatgataat-gaaa---tgagta
PAdxsdna	545	tggtgatcct---c---aacgacaac-gaca---tgtcga
LEdxsdna	719	ttgttatcct---a---aacgacaatagaca---agtttc
MTdxsdna	497	tgattatcgtggtc---aacgacaat-gggc---gcagct
RSdxs1dna	521	tcgtgatcct---g---aacgacaac-gaga---tgagca
RSdxs2dna	530	tcgtgatcct---g---aacgacaat-gaca---tgagca
SPCCdxsdna	503	tggtcgtgct---c---aacgacaat-gaca---tgtcga
ECdxsdna	524	tggtgattct---c---aacgacaat-gaaa---tgtcga
NMdxsdna	512	tggtcgtcct---c---aacgacaac-gaaa---tgtcga
HIdxsdna	524	tagttatttt---a---aatgataac-gaaa---tgtcta
SSdxsdna	500	tgatcatcgt---cgtcaacgacaac-gagc---gctcct
HPdxsdna	494	tcatgatttt---a---aacgataat-gaaa---tgagta
STdxsdna	732	tcgccccgccg-----gt---
CRdxsdna	770	tgcccacgcagtacaacaacaagaaccaggaccccg---
CJdxsdna	524	tttcaaaacca-----at---
PAdxsdna	575	tctcgcacaa-----gt---
LEdxsdna	750	tttacctactg-----ctact
MTdxsdna	530	acgcgcccaca-----at---
RSdxs1dna	551	tcgcgcgcgcg-----gt---
RSdxs2dna	560	tcgcgcgcgcc-----gt---
SPCCdxsdna	533	tctcgcccaac-----gt---
ECdxsdna	554	tttccgaaaat-----gt---
NMdxsdna	542	tttcccccaac-----gt---
HIdxsdna	554	tttcagaaaac-----gt---
SSdxsdna	533	acgcgcccacc-----at---
HPdxsdna	524	tcagcacgcct-----at---

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STdxsdna	745	--gggcgggccttctcgccctatcttgcgcgcctcatttcct
CRdxsdna	807	--gggcgcctgtccagcgccctggcgcgctgcaggcca
CJdxsdna	537	--tggagcaatttcaaagtatcttctcaggctatggcaa
PAdxsdna	588	--cggcgggctctccaactacctggcgaagatcctctcca
LEdxsdna	766	ctggatgggcccagttgctcctgttgagctctaagtagtg
MTdxsdna	543	--cgggggctgcgcgacctctggccacgctg-----
RSdxs1dna	564	--gggggcgctgtcgtcctatctctcgcggtc-tatgcg
RSdxs2dna	573	--gggggcgcttgcgcgctatctcgtgaatctc---tcct
SPCCdxsdna	546	--gggtgcgctctctcgtatct-----gaataagattcg
ECdxsdna	567	--cggcgcgctcaacaacctctggcacagctgctttcc-
NMdxsdna	555	--cgggtgcgttgcccaaataccttgccagc-----aacgt
HIdxsdna	567	--tgggtgcattaaataatcatcttgcgcg---tattttct
SSdxsdna	546	--cggcggcctcgccaaccacctggccacctgcgcacca
HPdxsdna	537	--tggagccttatccaaagcccttagccagctga--tgaa
STdxsdna	783	c--gtc-----cga-ata---t-----
CRdxsdna	845	a--ccg-----gcc-cct---g-----
CJdxsdna	575	c--gca-----gtt-tta---t-----
PAdxsdna	626	g--ccg-----cac-cta---t-----
LEdxsdna	806	c--tttgagcaggttacagtcta-ataggcct-----
MTdxsdna	574	c--ggc-----tgc-a-----
RSdxs1dna	601	g--gcg-----cgc-cgt---t-----
RSdxs2dna	608	c--gaa-----ggc-gcc---c-----
SPCCdxsdna	579	g--gtt-----ag-----
ECdxsdna	604	g--gta-----agc-ttt---a-----
NMdxsdna	588	c--gtg-----cgcgata---tg-----
HIdxsdna	602	ctggct-----ctc-ttt---a-----
SSdxsdna	584	c--cga-----cgg-cta---cgagaagggt
HPdxsdna	573	a--ggc-----ccg-ttt---t-----
STdxsdna	794	-ctcggc---c--tgc-gcga-gc--tcgcc-----
CRdxsdna	856	-cgcgag---c--tgc-gcga-ga--ttgcc-----
CJdxsdna	586	-caaagt---t--tta-aaaa-gcgtattgct-----
PAdxsdna	637	-agcagc---a--tgc-gcga-gg--gcagc-----
LEdxsdna	835	-ctcagagaac--taa-gaga-ag--tcgca-----
MTdxsdna	582	-gccggc---c--tac-gag-----c-----
RSdxs1dna	612	-ccagga---c--ttc-aaggcgg--ccgcc-----
RSdxs2dna	619	-ttegccacgc--tgc-gcgc-gg--ccgcc-----
SPCCdxsdna	585	--tgagc---cgatgc-agtt-gc--tcacc-----
ECdxsdna	615	-ctcttca--c--tgc-gcga-----
NMdxsdna	601	-cacgga---c--tgttgagt-ac--cgtca-----
HIdxsdna	615	-ctctacg--c--ttc-gtga-tg--gcagt-----
SSdxsdna	603	cctcgcc---t--ggg-gcaa-gg--acgtc-----
HPdxsdna	584	-accagt---c--ttt-ccgc-tc--taaagttaaaaa

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STdxsdna	815	-----aagcg-----cttcac-----cc-----
CRdxsdna	877	-----aagg-----cgtgac-----ca-----
CJdxsdna	610	-----aaaat-----gtt-----
PAdxsdna	658	-----aagaa-----ggt-----
LEdxsdna	859	-----aagg-----agttac-----ta-----
MTdxsdna	596	-----aggcg-----ctggagacgggcc-----
RSdxs1dna	634	-----aaggagcgctcggttctg-----cc-----
RSdxs2dna	643	-----gacgg-----gctcga-----gg-----
SPCCdxsdna	607	-----gatgg-----tttgac-----ccaggggat
ECdxsdna	630	-----aggcg-----ggaaaa-----aa-----
NMdxsdna	623	-----aagcg-----c-aaac-----gg-----
HIdxsdna	637	-----aaaaa-----aatc-----
SSdxsdna	625	-----ctgct-----gcgtac-----cc-----
HPdxsdna	613	atcttaagca-----ccttac-----ct-----
STdxsdna	828	gcaag-----ctttcg----cgccgc---c--tcaccgc
CRdxsdna	890	agcag-----ctgcct----gacgtt---g--tccagaa
CJdxsdna	618	ggata-----tatt-----gc---c--tgatagt
PAdxsdna	666	----g-----ctctcg----cgccgtg---c--ccggggc
LEdxsdna	872	agcag-----attggt---ggtcct---a--tgcatga
MTdxsdna	614	gcgac-----ctggtg----cgc-gc---g--gtgccgc
RSdxs1dna	657	cgaac-----cgttcc----aggaggcgc--gcgccgc
RSdxs2dna	656	cctcg-----ctgccg----gggccg---c--tccgcga
SPCCdxsdna	627	gcaacaaattcccttcgtcggcggcgc--cattacccaa
ECdxsdna	643	gtttt-----ctctgg----cgtgcc---g--ccaatta
NMdxsdna	635	gcaag-----gtatta----gacaaa---a--taccgcg
HIdxsdna	646	-cttg-----ataaag----ttcctc--caatcaaaaa
SSdxsdna	638	ccatc-----gtcggc---cacccc---c--tctacga
HPdxsdna	631	gaaag-----cgt-----ga---a--ttactta
STdxsdna	853	--g-gc---agccggcaaggcg---g-----aggaa--
CRdxsdna	915	--g-gc---aactgctaagatt---g-----acgag--
CJdxsdna	637	----gc---tacttatatggcc---a-----agcgt--
PAdxsdna	687	ctg-gg---agatcgcccggcgcaccg---aggaa--
LEdxsdna	897	--g-ct---tgctgcaaaagtt---g-----atgaa--
MTdxsdna	638	--ttgt---cggcggtctgtgg---t-----ttcga--
RSdxs1dna	685	--g-cc---a--aggagatgct---g-----aaga---
RSdxs2dna	681	--c-gg---ggcgcgccgggcg---c-----gccag--
SPCCdxsdna	664	--g-gctttgagccggttaag-g---a-----aggca--
ECdxsdna	668	--a-ag---agctgctcaaacgcaccg---aagaa--
NMdxsdna	660	--c-gc---gatggagtttgcc---c-----aaaaa--
HIdxsdna	672	--t-tt---tatgaaaaaacc---g-----aagaaca
SSdxsdna	663	--g-gc-----cctgcacggcg---ccaagaaggcg--
HPdxsdna	649	--g-cg---agtcg---tttt---g-----aagaa--

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STdxsdna	875	t-----tcgcccgcggcatg----g----cg-----
CRdxsdna	937	t-----atgctcgcggcatgatcag---cggc----
CJdxsdna	658	t-----ttgaagagagtttt----a-----aacttatt
PAdxsdna	715	t-----acgccaaggggcatg----c----tg-----
LEdxsdna	919	t-----atgctcgtggcatg----a----tt-----
MTdxsdna	661	t-----tctgcacagcgtc----a----ag-----
RSdxs1dna	704	-----gcgtc----a----cc-----
RSdxs2dna	703	c-----tcgtgaccgggatg----c----cg-----
SPCCdxsdna	689	tgaagcgcctctcctacagcaag-----
ECdxsdna	694	c-----atattaaaggcatg----g----ta-----
NMdxsdna	682	g-----tcgaacataaaaatc----a----aa-----
HIdxsdna	696	t-----atgaaaggtgtaat---gttttcg-----
SSdxsdna	688	t-----tcaaggacgccttc----g----cc-----
HPdxsdna	667	t-----ctttcaagctcat-----c-----
STdxsdna	893	a-----ccg-----gcggcacg-----
CRdxsdna	961	a-----ctg-----gctccacg-----
CJdxsdna	682	a-----ccc-----ctgggctt-----
PAdxsdna	733	g-----tcc-----ccggcacc-----
LEdxsdna	937	agtggttctg-----gatcaaca-----
MTdxsdna	679	g-----ccg-----gcatcaaggactcgtgtc
RSdxs1dna	712	g-----tcg-----gcggcacg-----
RSdxs2dna	721	g-----gcg-----ggggcacg-----
SPCCdxsdna	712	-----a-----ttggggcg-----
ECdxsdna	712	g-----tgc-----ctggcacg-----
NMdxsdna	700	a-----cccttgccgaagaagccgaaca-----
HIdxsdna	718	c-----cag-----aaagtaca-----
SSdxsdna	706	c-----cgc-----agggca-----
HPdxsdna	682	a-----ccc-----cgggcgtg-----
STdxsdna	905	-----c-----tgttcgagga
CRdxsdna	973	-----c-----tgtttgagga
CJdxsdna	694	-----t-----tgtttgaaga
PAdxsdna	745	-----c-----tgttcgagga
LEdxsdna	955	-----t-----tgtttgaaga
MTdxsdna	702	gccgcagttgc-----tgttcaccga
RSdxs1dna	724	-----c-----tcttcgagga
RSdxs2dna	733	-----c-----tcttcgagga
SPCCdxsdna	721	-----g-----tctttgaaga
ECdxsdna	724	-----t-----tgtttgaaga
NMdxsdna	723	-----cgccaaacagtgcactgtctttgtttgaaaa
HIdxsdna	730	-----t-----tatattgaaga
SSdxsdna	716	-----tgttcgagga
HPdxsdna	694	-----t-----tttttgaaga

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STdxsdna	916	acttggcttctattatgtcggcccgatcgacggccacaat
CRdxsdna	984	gctgggcctgtactacatcgccctgtggacggccacaac
CJdxsdna	705	attaggccttgaatatatagggcctattgatggacataat
PAdxsdna	756	gctcggctggaattacatcgggcccgatcgacggccacgac
LEdxsdna	966	acttggacttttactatattggctcctgtggatgggtcacaac
MTdxsdna	723	cctcgggttgaagtacgtcggcccggtcgacggcca---t
RSdxs1dna	735	gctgggtttctcctatgtcggcccgatcgacgggcacgat
RSdxs2dna	744	gctgggcttcacctatgtcggcccatcgacggccacgac
SPCCdxsdna	732	gctgggcttcacctacatggggccagtggtgggtcacaac
ECdxsdna	735	gctgggctttaactacatcgggcccggtggacgggtcacgat
NMdxsdna	753	cttcgggttccgctataccggcccggtggacggacacaac
HIdxsdna	741	actcgggtttaactatattggcccgatggatgggcataac
SSdxsdna	726	cctgggcctgaagtacgtcggcccatcgacgggcacgac
HPdxsdna	705	attaggcattaactatatagggcctattaatgggc-----
STdxsdna	956	ctcgagcatctgatcccgggtgctggagaatgtcc-g----
CRdxsdna	1024	ctggacgacctcatcgccgtgctcagcgaggtgc-g----
CJdxsdna	745	ttaggtgaaattat-----ttctgcattaaaacaag----
PAdxsdna	796	ctgccgacctggtgggtaccctgcgcaacatgc-g----
LEdxsdna	1006	attgatgatctaattgctgattctcaaagaggtta-gaagt
MTdxsdna	760	gacgag-----cgggcggtggaggtcgcgct-----
RSdxs1dna	775	ctcgaccagcttctgcgggtgctgcggaccgtca-a----
RSdxs2dna	784	atggaggcgctcctccagacgctgcgcgcggcgc-g----
SPCCdxsdna	772	cttgagaactgatc-----gccaccttcc-g----
ECdxsdna	775	gtgctggggcttatcaccacgctaaagaacatgc-g----
NMdxsdna	793	gtcgaaaatctggtcgatgtattggaagacctgc-g----
HIdxsdna	781	attgatgaattagtggctacgcttacgaatatgc-g----
SSdxsdna	766	atcggcgcggctcgagtcgcgcgctgc-----gcc-g----
HPdxsdna	740	----atgatttgagcgcgattattgaaaccttaa-a----
STdxsdna	991	-c---gaca---gcga-gc---a---g-----ggc---
CRdxsdna	1059	-c---agcg---ccga-ga---ccgtg-----ggc---
CJdxsdna	776	-c---aaaa---gctatgc---a---a-----aag---
PAdxsdna	831	-c---gaca-----t-ga---a---g-----ggc---
LEdxsdna	1045	ac---taaa---ac-a-ac---a---g-----gtc---
MTdxsdna	786	-g---cgca---gcgc-gc---g---g-----cgcttc
RSdxs1dna	810	-g---cagc---gggc-gc---a---t-----gcg---
RSdxs2dna	819	-g---gccc---g-ga-cc---ac-g-----ggg---
SPCCdxsdna	798	-c---ga-a---gcgc-ac---a---aacacaccgga---
ECdxsdna	810	-c---ga-----cct-ga---a---a-----ggc---
NMdxsdna	828	-c---ggac---gc---a---a---a-----ggc---
HIdxsdna	816	-----ta---atct-ga---a---a-----ggc---
SSdxsdna	795	-c---gccaaagcgctt-cc---a---c-----ggg---
HPdxsdna	771	-attagcca---aaga-gcttaa---a-----gag---

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STdxsdna	1007	-----ccgatcctgatccatgtcgtgaccaagaagggca
CRdxsdna	1078	-----ccggtgctggtgcacgtggtaacggagaagggcc
CJdxsdna	793	-----ccttggtgatacatgctcaaaccataaagggtg
PAdxsdna	844	-----ccgcagttcctccatgtggtgaccaagaaaggca
LEdxsdna	1061	-----cag-tactgatccatgttgtcactgagaaaggca
MTdxsdna	805	ggtgcaccggtgatcgtgcacgtcgtcaccgcgaagggca
RSdxs1dna	826	-----ccggtgctgatccatgtcatcaccaagaagggca
RSdxs2dna	835	-----ccggtgctcatccatgtggtcacgaagaagggca
SPCCdxsdna	820	-----ccagtactcgtccacgttgccacaaccaagggtg
ECdxsdna	823	-----ccgcagttcctgcatatcatgacaaaaaagggtc
NMdxsdna	841	-----ccgcagcttctgcacgtcatcacaaaaaagggtg
HIdxsdna	829	-----ccacaatttttgcataataaaaacgaaaaaagggtg
SSdxsdna	814	-----ccggtgctggtgcactgcctcacgtcaagggcc
HPdxsdna	793	-----ccggtgctaataccatgcgcaaaccttaagggtg
STdxsdna	1041	agggctatgccccggccgaagcg---gcggcggacaagta
CRdxsdna	1112	gcggtacctgcccggcgagacg---gcgcaggacaagat
CJdxsdna	827	aaggctatgcttttagctgaagga---aaacatgctaaatg
PAdxsdna	878	agggcttcgccccggccgaactg---gatccgatcggcta
LEdxsdna	1094	gaggttatccatattgctgagaga---gctgcagataagta
MTdxsdna	845	tgggctacccggccggccga-----ggccgac-----
RSdxs1dna	860	ggggctatgctccggccgaggcc---gcgcgcgacccgtgg
RSdxs2dna	869	agggttacgccccggcgagaat---gccccgacaagta
SPCCdxsdna	854	agggctatccctacgctgaagaa---gatcaggttggcta
ECdxsdna	857	gtggttatgaaccggcagaaaaa---gaccgatcacttt
NMdxsdna	875	acggctacaaaactcgccgaaaaac---gatcccgctcaata
HIdxsdna	863	aaggatacgacaccgcagaaaaa---gatccgatttggttt
SSdxsdna	848	gcggtacgaaccggccctcgcccacgaggaggaccactt
HPdxsdna	827	aaggctataagatcgctgaaggg---cgctatgaaaaatg
STdxsdna	1078	tcacggcgctccagaag--tt--cgacgt----gacg-acc
CRdxsdna	1149	gcacggtgtggtcaag--tt--cgaccc----ccgc-acc
CJdxsdna	864	gcacggggtgggagcc--tt--tgatat----agat-agt
PAdxsdna	915	ccacgcgatcaccaag--ct--gga-----agc-tcc
LEdxsdna	1131	tcatggagttgccaag--tt--tgatcc----agca-aca
MTdxsdna	871	-caggccgagcagatgcatt--ccacggtcccgatcgatc
RSdxs1dna	897	ccatgccacgaacaag--tt--caacgt----cctg-acc
RSdxs2dna	906	tcacggggtgaacaag--tt--cgaccc----cgtc-acg
SPCCdxsdna	891	tcatgccccaaaatccc--tt--tgatct----ggcg-aca
ECdxsdna	894	ccacgcgctgcctaaa--tt--tgatcc----ctcc-agc
NMdxsdna	912	ccacgcgctgcctaac--ctgcctaaag----aaag-cgc
HIdxsdna	900	ccacggtgtacctaaa--tt--tgatcc----aatc-agt
SSdxsdna	888	ccacaccgtcggcggtg--at--ggaccc----gctc-acc
HPdxsdna	864	gcatggggtggggcct--tt--tgattt----ggat-acc

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STdxsdna	1109	ggggcacaggcc-----aaggcacc-----ccgggcc-
CRdxsdna	1180	ggcaagcaggtg-----caggccaag-----acgaagg-
CJdxsdna	895	ggagagagtgtt-----aaaaaaagt-----gatacta-
PAdxsdna	942	cggcagtgcgcc-----gaagaagac-----c--ggcg-
LEdxsdna	1162	ggaaagcaattc-----aaag-----c-----cagtgcc-
MTdxsdna	908	cggccaccggac-----aagccacca-----aggtggc-
RSdxs1dna	928	ggcgcgcaggtg-----aagccggtc-----tcgaacg-
RSdxs2dna	937	ggcgagcagaag-----aagtcggtg-----gccaacg-
SPCCdxsdna	922	ggg---aaggct-----aaaccagcttcaaaaccgaagc-
ECdxsdna	925	ggttggttgccg-----aaaagtagc-----ggcggtt-
NMdxsdna	945	ggcgcaaattgccgtctgaaaaagaac-----ccaagcc-
HIdxsdna	931	ggcgaaattgccc-----aa---aaac-----aatagta-
SSdxsdna	919	tgtg---agccc-----ctctcgccc-----accgacg-
HPdxsdna	895	gg---cttgtct-----aaaaaatcc-----aaaag---
STdxsdna	1137	---cgccc--gc-----ctat-----accaaggtgtt
CRdxsdna	1208	---ccatg--tc-----gtac-----acgaactactt
CJdxsdna	923	---aaaaa--tc-----tgct-----actgaaatttt
PAdxsdna	968	---gaccc--aa-----gtat-----tccagcgtctt
LEdxsdna	1187	agacacag--tc-----ctat-----acaacatattt
MTdxsdna	936	---cggcccagg-----ctgg-----acggcgacctt
RSdxs1dna	956	---ccccc--tc-----ctat-----accaaggtctt
RSdxs2dna	965	---cgccg--aa-----ctac-----accaaggtctt
SPCCdxsdna	953	---cgct--ag-----ctat-----tccaaagtgtt
ECdxsdna	953	---tgccg--ag-----ctat-----tcaaaaatctt
NMdxsdna	978	---cgccg--ccaaaccgacctat-----acccaagtgtt
HIdxsdna	956	---aacca--ac-----ttat-----tcgaaaatttt
SSdxsdna	944	---gcccg--tc-----ctgg-----acctcggtgtt
HPdxsdna	918	---cgcaa--tc-----ttatcgcccactgaagcgta
STdxsdna	1159	cgccgatgcgctgctcgc-cgaagcgg-agcgtgatgcgt
CRdxsdna	1230	cgcgacgcgctgacggc-ggaggcgg-agcgcgacagcc
CJdxsdna	945	ttctaagaatttgcttga-tttagcct-caaaatatgaaa
PAdxsdna	990	cggccagtggctgtgcga-catggccg-cccaggacgcg-
LEdxsdna	1212	tgccgaggctttaattgc-agaagcag-aagcagataaag
MTdxsdna	960	ctctgatgcacttatcgg-ctacgc-----ccagaaacgc
RSdxs1dna	978	cgcccagagcctcatcaa-ggaggccg-aggtcgacgagc
RSdxs2dna	987	cggctccaccctgaccga-ggaggccg-cgcgcgatccgc
SPCCdxsdna	975	tggccaaaccctgacgac-cttgcca-agagcgat-cgc
ECdxsdna	975	tggcga----ctggttgctgcgaaacggcagcgaaagacaa
NMdxsdna	1008	cggcaaattggctgtgcga-ccgggagg-cggcagattc--
HIdxsdna	978	tggcgattggctatgtga-aatggcag-aaaaagatgcc-
SSdxsdna	966	cggcgacgagatcgt--a-cggatcgg-cgcggagcgcga
HPdxsdna	945	ttctaacacccttttaga-attagcta-aaaaagatgaaa

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STdxsdna	1197	cgg----tctg-c--gcg--atcaccgcggcgatgccctc
CRdxsdna	1268	gca----tcgt-g--gcg--gtgcacgcggccatggcg
CJdxsdna	983	ata----ttgt-t--ggg--gttacggcggtatgccaa
PAdxsdna	1027	-cg----cctg-c--tcggcatcaccgcggcgatgaagga
LEdxsdna	1250	aca----ttgt-t--gca--atccatgctgcatggggg
MTdxsdna	994	cgtgacatcgt-g--gcc--attaccgcggccatgccgg
RSdxs1dna	1016	gga----tctg-c--gcg--gtgacggccgcatgccgga
RSdxs2dna	1025	gca----tcgt-g--gcg--atcaccgcggcatgccctc
SPCCdxsdna	1012	cgc----attgtc--ggg--attacggctgcgatggcgac
ECdxsdna	1011	caa----gctg-atggcg--attactccggcgatgcgtga
NMdxsdna	1044	ccg----actg-gttgcg--attaccccgccatgcgcga
HIdxsdna	1016	aaa----ttat-a--ggt--atcacacctgcaatgcgtga
SSdxsdna	1002	ggaca--tcgt-c--gcg--atcaccgcggcgatgctc--
HPdxsdna	983	aaa----tcgt-a--ggc--gtaaccgcggcgatgcctag
STdxsdna	1228	gggcacc-----gggctcg-acaagttccaggcgacg--t
CRdxsdna	1299	cggcacc-----ggcctgt-accggttcgagaagaag--t
CJdxsdna	1014	tggaca-----ggtcttg-ataagcttatagaaaaa--t
PAdxsdna	1059	aggttcc-----gacctgg-tggcctt-cagcgaacg--t
LEdxsdna	1281	tgggacc-----ggaatga-accttttcca-tcgtcg--c
MTdxsdna	1029	ccccacc-----gggctga-ccgcgttcgggcagcgc--t
RSdxs1dna	1047	cgggacg-----gggctca-acctcttcggcgagcgg--t
RSdxs2dna	1056	gggcacc-----ggcgtcg-acatcatgcagaagcgt--t
SPCCdxsdna	1044	aggcacc-----ggcttgg-acattctccagaagcg--c
ECdxsdna	1044	aggttcc-----ggcatgg-tcgagttttcacgtaaa--t
NMdxsdna	1077	gggcagc-----ggcttgg-ttgagtttgga-acaacga-t
HIdxsdna	1047	gggttca-----ggtatggtagaattttc--ccaacgc-t
SSdxsdna	1033	---cacccgggtggggtcg-ccaggttc--gccgaccgct
HPdxsdna	1014	cggcaca-----ggattag-acaaactcattgacgct--t
STdxsdna	1260	tccccg-atc-gcaccttcgacgtcgctatcgccgagcag
CRdxsdna	1331	tccccg-acc-gcacctttgacgtgggcattgcggagcag
CJdxsdna	1046	atccaa-atc-gtttttgggatgtggctattgcagaacag
PAdxsdna	1090	tatccggaac-gctacttcgacgtcgccatcgccgaacag
LEdxsdna	1312	ttccca-acaaggtgttttgatgttggaatagcagaacaa
MTdxsdna	1061	tccccg-atc-gattgttcgacgtcgggatcgccgagcaa
RSdxs1dna	1079	ttccga-agc-gcaccttcgatgtgggcatcgccgaacag
RSdxs2dna	1088	tcccga-acc-gcgtcttcgacgtgggcatcgccgagcag
SPCCdxsdna	1076	tgccga-agc-aatacatcgatgttggcattgccgaacag
ECdxsdna	1076	tccccg-atc-gctacttcgacgtggcaattgccgagcaa
NMdxsdna	1109	tccccg-acc-gctatttcgatgtcggcatcgccgagcag
HIdxsdna	1079	ttccaa-aac-aatattttgacgtagcgattgcagaacag
SSdxsdna	1067	tccccg-acc-gggtctgggacgtcggcatcgccgagcag
HPdxsdna	1046	accctt-tgc-gcttttttgatgtcgctatcgctgagcaa

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STdxsdna	1298	cacgcggtcacct-tcgcagcgggccttgccgcgcagggg
CRdxsdna	1369	cacgccgtgacct-ttgctgccggcctggcgtgcgagggc
CJdxsdna	1084	catgcagtaactt-ctatggccgctatggcaaaagaagga
PAdxsdna	1129	catgccgtgaccc-tggccgcggcatggcctgcgagggc
LEdxsdna	1351	catgcagtaacct-ttgctgctggattggcctgtgaaggc
MTdxsdna	1099	cacgcgatgacgt-cggcggcgggttgccgatgggtggg
RSdxsldna	1117	catgcggtgacct-tctcggcggcgcttgccgcaggcggc
RSdxs2dna	1126	catgccgtgacct-tcgcggcggcctgcgggggcggg
SPCCdxsdna	1114	cacgccgtggtgc-tagctgccggtatggcctgcgatggc
ECdxsdna	1114	cacgcggtgacct-ttgctgcgggtctggcgattgggtggg
NMdxsdna	1147	cacgccgttacct-ttgccggcggtttggcctgcgaaggg
HIdxsdna	1117	cacgctgtcacgt-ttgccacaggacttgcaattggcgga
SSdxsdna	1105	cacgcggccgtgt-cgcggccgggctgccaccggcgga
HPdxsdna	1084	cacgctttaacttctagcagc--gctatggctaaagaggg
STdxsdna	1337	atgcggccggttctgcgcg-atctactcgaccttctcgag
CRdxsdna	1408	ctggtgcccttctgcacc-atctacagtaccttcatgcag
CJdxsdna	1123	tttaaacccttttattgca-atatatagcacctttttgcag
PAdxsdna	1168	atgaagccggtggttagcg-atctactcgaccttctccag
LEdxsdna	1390	attaaacccttctgtgca-atctattcgtctttcatgcag
MTdxsdna	1138	ctgcaccccggtggtggcg-atctactcgacgttctgaac
RSdxsldna	1156	atgcggcccttctgcgcc-atctattccaccttctccag
RSdxs2dna	1165	atgaagcccttctgcgcg-atctattcctcgttctgcaa
SPCCdxsdna	1153	atgcgtccggtggtggca-atctattccaccttctgcag
ECdxsdna	1153	tacaaaccattgtgcgcg-atctactccactttctgcaa
NMdxsdna	1186	atgaagcccgctcgtggcg-atctattccacctttttacaa
HIdxsdna	1156	tataaacctgtcgtcgca-atctactcgacatttttacia
SSdxsdna	1144	ctgcacccggtcgtcgcc-gtctacgccaccttctcaac
HPdxsdna	1122	gtttaaacccttttgtagcatctattctacttttttgag
STdxsdna	1376	cgcgcttacgaccaggtcgtccacgacgtcgcgatccaga
CRdxsdna	1447	cgcggttacgaccagatcgtgcacgacgtgtccctgcaga
CJdxsdna	1162	cgtgcttatgatcaagtgatccatgattgtgcgattatga
PAdxsdna	1207	cgcgcttacgaccagttgatccatgacgtcgcggtgcagc
LEdxsdna	1429	agggcttatgaccaggtagtgcacgttgatttgcaaa
MTdxsdna	1177	cgggcgttcgaccagatcatgatggatgtggcgctgcaca
RSdxsldna	1195	cgcggttacgaccagatcgtgcacgtggcaatccagc
RSdxs2dna	1204	cgggggttacgaccagatcgcccatgacgtggcgctgcaga
SPCCdxsdna	1192	cgggcctttgatcaagtcatccacgacgtttgtatccaaa
ECdxsdna	1192	cgcgcttatgatcaggtgctgcacgtggcgattcaaaa
NMdxsdna	1225	cgcgcttacgaccaactggtgcacgacatcgccctgcaaa
HIdxsdna	1195	cgtgcttacgatcaattaattcacgatgttgccattcaaaa
SSdxsdna	1183	cgcgcttcgaccagctcctgatggacgtcgc--cctgc
HPdxsdna	1162	agggcttatgattctattgtgcacgttgattttcta

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STdxsdna	1416	acc--tgccg-gtccgcttcgcatcgaccgcgcgggcct
CRdxsdna	1487	agc--tgccct-gtgcgcttcgctatggaccgcgcgtggcct
CJdxsdna	1202	att--taaat-gtggtttttgctatggatagggcagggat
PAdxsdna	1247	acc--tcgac-gtgctgttcgccatcgaccgcgcggcct
LEdxsdna	1469	agc--tgccc-gtgaggtttgcaatggacagagcaggtct
MTdxsdna	1217	agc--tgccg-gtcaccatggtgctggaccgtgccgggat
RSdxs1dna	1235	gcc--tgccg-gtgcgctttgccatcgaccgcgcgggcct
RSdxs2dna	1244	acc--ttccc-gtccgcttcgctgatcgaccgggcggggct
SPCCdxsdna	1232	agc--tgccc-gtcttcttctgcctcgatcgcgcggggat
ECdxsdna	1232	agc--ttccg-gtcctgttcgccatcgaccgcgcgggcat
NMdxsdna	1265	acc--tgccc-gttttgtttgccgtcgaccgcgcgggcat
HIdxsdna	1235	atc--tccct-gtgctatttgcaattgatcgagcagggat
SSdxsdna	1220	accgctgcggtgtgaccttcgtcctggaccgggcggcgct
HPdxsdna	1202	gct--tgccg-attaaattagccattgacagggctgggat
STdxsdna	1453	ggtcggtgcccagcggcgccgacccatgccggcagcttcgac
CRdxsdna	1524	ggtgggcgctgacggctccacgcactgccggcgcccttcgac
CJdxsdna	1239	agtaggcgaagatggggagacgcatcaagggtgtttttgat
PAdxsdna	1284	ggtcggcgaggacggcccgacccacgccggtagcttcgac
LEdxsdna	1506	tggtggagcagatggtccaacacattgtggtgcattttgat
MTdxsdna	1254	caccggtagcgacggcgccagccacaacggaatgtgggac
RSdxs1dna	1272	cgtgggggcccagcggcgccacccatgcccggctcgttcgat
RSdxs2dna	1281	cgtgggggcccagatggcgcgacccatgcccgggccttcgat
SPCCdxsdna	1269	agttggcgcggtgacggcgcgactcaccaaggcatgtacgac
ECdxsdna	1269	tggtggtgctgacgggtcaaaccatcagggtgcttttgat
NMdxsdna	1302	cgtcggcgcgacggcccgacccatgccgggtttgtacgat
HIdxsdna	1272	agtgggtgcagatggggctacacatcaagggtgcattcgat
SSdxsdna	1260	cacgggcccgcgacggcgccctcgcacaaaggcatgtgggac
HPdxsdna	1239	tgtgggccaagatggcgagacgcaccaagggcttttagac
STdxsdna	1493	gtgacctatctcgccagcctgcccattttcgtgggtgatgg
CRdxsdna	1564	gtgacgttcatggcgtcgctgccgcacatgatcaccatgg
CJdxsdna	1279	cttagtttttttagctcctttgccaaatttcactcttttag
PAdxsdna	1324	atctcctacctgcgctgcacccccggcatgctggtgatga
LEdxsdna	1546	gttacttacatggcatgtcttcctaacatgggttgtaatgg
MTdxsdna	1294	ttgtcgatgctgggtatcgtgcccgcatccgggtggcag
RSdxs1dna	1312	gtggccttcctgtcgaacctgcccgcatcgtggtgatgg
RSdxs2dna	1321	gttggcttcactccttcgctgcccaacatgaccgtgatgg
SPCCdxsdna	1309	attgcttacctgcggtgatcccaacatgggtgctgatgg
ECdxsdna	1309	ctctcttacctgcgctgcataccggaaatgggtcattatga
NMdxsdna	1342	ttaagctttttgcgctgcattccgaat---atgattgtcg
HIdxsdna	1312	attagctttatgcgttgcatccaaatatgatcattatga
SSdxsdna	1300	atgtccgtcctccaggctcgtgcc---ggcctcaggatcg
HPdxsdna	1279	gtgtcgtattttgcgctctatccctaa---catggtcattt

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STdxsdna	1533	cggccgcggacgaggtcgag-ctcgtccacatg--accca
CRdxsdna	1604	ctccctcgaacgaggcgag-ctcatcaacatg--gtggc
CJdxsdna	1319	c-----cccaagagat-----gaacaaatg--atgca
PAdxsdna	1364	ccccagcgcgacgaggacgag-ctgcgcaagctg--ctcac
LEdxsdna	1586	ctccttctgatgaagcgag-ctatttcacatggtagcaa
MTdxsdna	1334	cgcccagagacg-----cca-cccggttgcgtg--aagaa
RSdxs1dna	1352	ccgcccgcgacgaggccgag-ctcgtccatg--gtagc
RSdxs2dna	1361	ccgcccgcgacgaggccgag-ctcatccacatg--atcgc
SPCCdxsdna	1349	caccgaaagatgaggccgaa-c---tgcagcgg--atgct
ECdxsdna	1349	cccgagcgcgtgaaaacgaa-tgtcgccagatg--ctcta
NMdxsdna	1379	ccgcccgcgagcgatgaaaat-gaatgccgcctg--ctgct
HIdxsdna	1352	cgccgagtgcgtgaaaatgaa-tgccgtcaaag--ctcta
SSdxsdna	1337	ccgccccgcgcgcgcgcgcgcac-cacgtgcgcgcc--cagct
HPdxsdna	1316	ttgccccacgcagacaatgagactttaaaaaacg--ccgtg
STdxsdna	1570	ca-cg---gcg--g--cga--tg--cacg-----acag
CRdxsdna	1641	ca-cctgcgcc--g--cca--tc--gacg-----ac--
CJdxsdna	1344	aa-at---ata--a--tgg--ag--tatgcttatttacat
PAdxsdna	1401	ca-c-----cg--g--ctacctg--ttcg-----a---
LEdxsdna	1625	ctgct---gcc--g--cca--tt--gatg-----aca-
MTdxsdna	1366	ct-cg---gcgagg--cgc--tc--gacgtcg----acga
RSdxs1dna	1389	ca-cc---gcc--g--ccg--cc--catg-----acga
RSdxs2dna	1398	ca-cc---gcc--g--tgg--cc--ttcg-----gcga
SPCCdxsdna	1383	ag-tg---acg--g--gta--tt--gaat-----acga
ECdxsdna	1386	ta-c-----cg--g--cta--t---cact-----ataa
NMdxsdna	1416	tt-cg---acc--t--gct--at--cagg-----caga
HIdxsdna	1389	ta-ca---ggt--tatcaa--tg--tgga-----aaac
SSdxsdna	1374	gc-gg---gag--g--cgg--tc--gccg-----tgga
HPdxsdna	1354	cg-tt---ttg--c--caa--tgaacacg-----attc
STdxsdna	1591	c--g---gcccgatcgcgctgc-gctatccacgcggcaac
CRdxsdna	1663	-----gcgccctcgtgcttccgcttccccgcggcaac
CJdxsdna	1372	caag---gacctattgctttgc-gttatcctag-----ag
PAdxsdna	1419	t--g---gcccgccgcggtgc-gctatccgcgcggcagc
LEdxsdna	1646	-----gaccaagtgtttta-gatacccaagaggaaat
MTdxsdna	1392	c--g---gcccgcgcggttac-ggttccc-----caaa
RSdxs1dna	1410	a--g---ggcccatcgccttcc-gctatccgcgcggcgac
RSdxs2dna	1419	g--g---gccccatcgccttcc-gcttcccgcggggcgag
SPCCdxsdna	1404	c--g---gcccgatcgcctatgc-gtttcccgcgcgggaat
ECdxsdna	1404	c--gatggcccgctcagcgggtgc-gctaccgcgctggcaac
NMdxsdna	1437	c--g---cgcccgcgcgcgtcc-gctatccgcgcggcacg
HIdxsdna	1412	c--t---gc-----ggcagtg-cgtaccctcgcggaaat
SSdxsdna	1395	c--g---acgcgcgcgcgcgtg-----atccgcttcccgaa
HPdxsdna	1377	a--a---gcccttgccgcgttcc-gatacc-----ctag

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STdxsdna	1625	ggcg---tcggactggc-gctgccc-----
CRdxsdna	1696	ggcc---tgggcctgga-cctggcgcctacggcatcagc
CJdxsdna	1403	ggag---ttttattttg-gataaag-----
PAdxsdna	1453	ggcc---ccaaccatcc-gatcgat-----
LEdxsdna	1678	ggga---tcggtgtaga-gcttccg-----
MTdxsdna	1420	ggtgatgtgggagaaga-tatttc-----
RSdxsldna	1444	ggcg---tgggggtcga-ggtgccg-----
RSdxs2dna	1453	gggg---tgggcgtcga-gatgccc-----
SPCCdxsdna	1438	ggta---ttggcgtaacc-cctgccggaag-----
ECdxsdna	1441	gcgg---tcggcgtgga-actg-----
NMdxsdna	1471	ggta---cgggcgtgcc-ggtttca-----
HIdxsdna	1441	gccg---ttggtgtaaa-act---t-----
SSdxsdna	1425	ggag---tccgtcggcccgcgatc-----
HPdxsdna	1404	gggg---tcg---tttgc-gttaaaa-----
STdxsdna	1646	aa-gg---t---tccggag-----c-----ggctg-----
CRdxsdna	1732	aa-gg---a---cctgaag-----g-----gtgtgcccct
CJdxsdna	1424	aa-tt---taatccttgt-----g-----agata-----
PAdxsdna	1474	cc-gg---a---cctgcaa-----c-----cggtg-----
LEdxsdna	1699	gctgg---a---aacaaggaattc-----ctctt-----
MTdxsdna	1443	---gg---c---tttggag-----c-----ggcgt-----
RSdxsldna	1465	gt-ga---a---gggcgtg-----c-----cgctc-----
RSdxs2dna	1474	ga-gc---g---cgggacg-----g-----tgctg-----
SPCCdxsdna	1463	aa-gg---c---tg-ggag-----t-----cgctc-----
ECdxsdna	1459	ac-gc---c---gctggaa-----a-----aacta-----
NMdxsdna	1492	ga-cg---g---catggaa-----a-----ccgtg-----
HIdxsdna	1459	ac-tc---c---tttagaa-----a-----tgctt-----
SSdxsdna	1447	cc-gg---c---cctcgac-----c-----gggtc-----
HPdxsdna	1423	ga-gggggt---ttttgag-----cctagcgggttt-----
STdxsdna	1664	-gaaatcggcaagggtc--gcgtggtccga----gag---
CRdxsdna	1755	cgaggtgggcaagggtg--ttgtccgccgc----cag---
CJdxsdna	1444	-aaacttggttaagg-----cac----aat---
PAdxsdna	1492	-gagatcggcaagg-----gcgtggtccgt---cggcgc
LEdxsdna	1723	-gaggttggttaaaggta--ggatattgatt---gag---
MTdxsdna	1459	-ggaggcgtggatgtgctggcgccgcccgc--gat---
RSdxsldna	1483	-cagatcggccgtggcc--gggtggtgagc---gag---
RSdxs2dna	1492	-gagcccgccggggcc--gcgtggtgcgc---gaa---
SPCCdxsdna	1480	-ccgattgggaaagcag--agcaactgcgc---caa---
ECdxsdna	1477	-ccaattggcaaaggca--ttgtgaagcgt---cgt---
NMdxsdna	1510	-gaaatcggcaagggtc--ttatccgccgc---gaa---
HIdxsdna	1477	-cctattggtaaatcac--gtttaattcga----aaa---
SSdxsdna	1465	-g---gcggcctcgatg--tgctgcaccgc---ga---
HPdxsdna	1450	-gttttaggccaaag-c--gaattgttgaaaaagag---

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STdxsdna	1694	ggcaagaaggtagcgatcctgtcgctcggcacg-cgcctt
CRdxsdna	1786	ggcaaggacgtgtgcctgggtggcgtagcgacg-agtggtg
CJdxsdna	1463	ggcttgtaaaaaataatagtgaatt-----g-cttttt
PAdxsdna	1522	ggcggcagggtcgcactgctggtcttcggcggtg-cagttg
LEdxsdna	1753	ggggagagagtggtctattgggatatggctc--agcagt
MTdxsdna	1492	ggtttgaaccacgacgtcctggttggtggccatc-ggc---
RSdxs1dna	1513	ggcacgcgaatcgcgctcctgtccttcgggcacc-cgtctg
RSdxs2dna	1522	gggacggatgtcgcgatcctctccttcggcgcg-catctg
SPCCdxsdna	1510	ggcgatgatttgctgatgttggttacggctcg-atggtc
ECdxsdna	1507	ggcgagaaaactggcgatccttaactttggtacg-ctgatg
NMdxsdna	1540	ggtgagaaaaccgcattcattgccttcggcagt-atggtc
HIdxsdna	1507	ggtcaaaaaattgcgatttttaaattttggtact-ctatta
SSdxsdna	1491	--cgagcggccccgaggtgctgctggtcgccgtg-ggcgtc
HPdxsdna	1483	ggcgaaattttactcat--aggctatggtaatggcggtggg
STdxsdna	1733	gcgg--aagca-----ctaa-aggcc-----gcc
CRdxsdna	1825	aacg--aggcg-----ctgg-ccgcg-----gcg
CJdxsdna	1496	taggttatgga-----caag-gtgtg-----gca
PAdxsdna	1561	gcgg--aggcg-----atga-aggtc-----gcc
LEdxsdna	1791	gcag--aactg-----tttgatgct-----gct
MTdxsdna	1528	gcgt--tcgca-----ccga-tggcggttgggcggtggcc
RSdxs1dna	1552	gccg--agggtg-----cagg-tggcc-----gcc
RSdxs2dna	1561	cacg--aggcc-----ttgc-aggcg-----gcg
SPCCdxsdna	1549	tatc--cggcc-----ctgc-agacg-----gca
ECdxsdna	1546	ccag--aagcg-----gcga-aagtc-----gcc
NMdxsdna	1579	gccc--ctgca-----ttgg-cggtc-----gcc
HIdxsdna	1546	ccat--ccgct-----ttag-agtta-----tca
SSdxsdna	1528	atgg--ca-caggtctgcctcc-agacc-----gcc
HPdxsdna	1521	gcgg--gcgca-----ttta-----g-----tcc
STdxsdna	1754	gacacgctcgaggcc--aagggcctctcgaccaccg----
CRdxsdna	1846	gacatgctggagcg--gatggcggtgtccaccaccg----
CJdxsdna	1519	aaagcgtggcaagtcttaagagccttgcaagaaatgaata
PAdxsdna	1582	gaaagcctcgacg-----ccacgg----
LEdxsdna	1813	attgtgctagaatcc--cgcggttacaagtaacag----
MTdxsdna	1558	aagcggctgcacaac--caggggatcggtgtgacgg----
RSdxs1dna	1573	gaggcgctggctgcg--cgcggtatctctccacgg----
RSdxs2dna	1582	aaacttctcgaggcc--gagggggtgagcgtgaccg----
SPCCdxsdna	1570	gaactgctgaatgag--cacggcatctcagctactg----
ECdxsdna	1567	gaatcgctgaacg-----ccacgc----
NMdxsdna	1600	ggaaaactgaacg-----ccaccg----
HIdxsdna	1567	gaaaaactcaatg-----caacgg----
SSdxsdna	1555	gagctgctccggggcc--cgcgcatcggtgacgg----
HPdxsdna	1538	aactggctttaaaag--aaaaaacatagaatgcgc----

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STdxsdna	1788	-----tcgcc--gacctgcgcttcgccaaaccg
CRdxsdna	1880	-----tcatt--gacgcgcgcttctgcaagcct
CJdxsdna	1559	ataatgctaatttgatt--gatttaatttttgctaaacct
PAdxsdna	1601	-----tcgtc--gacatgcgtttcgtcaaacc
LEdxsdna	1847	-----ttgca--gatgcacgtttctgcaaacca
MTdxsdna	1592	-----tgatc--gaccgcgcgtgggtgttgccg
RSdxs1dna	1607	-----ttgcg--gatgcgcgctttgcaaagccg
RSdxs2dna	1616	-----tggcc--gacgccgcgttctcgcgcgccg
SPCCdxsdna	1604	-----tgatc--aatgccgcgttcgccaaagccc
ECdxsdna	1586	-----tggtc--gatatgcgttttgtaaaccg
NMdxsdna	1619	-----tcgcc--gatatgcgcttcgtcaaaccg
HIdxsdna	1586	-----ttgtc--gatatgcgttttgtaaaccg
SSdxsdna	1589	-----tcgtc--gaccgcgcgtgggtcaagccc
HPdxsdna	1572	-----tctcttgatctcagggtttttaagcct
STdxsdna	1814	ctcgacgaggatctgatcc-gc-c-gcctgctcaccaccc
CRdxsdna	1906	ctggacaccaagctgatcc-gctc-ggctgc-caaggagc
CJdxsdna	1597	ttagatgaagagcttttgt-gt-gagcttgctaaaaaag
PAdxsdna	1627	ctcgacgaagccctggtac-gc-g-aattggcgggcagcc
LEdxsdna	1873	ctggaccatgccctcataa-gg-a-gccttgcaaaatcac
MTdxsdna	1618	gtgtctgacggtgtg---c-gc-g-aactggcgggtgcagc
RSdxs1dna	1633	ctcgaccgggatctgat----c-c-tgcagctcgcggccc
RSdxs2dna	1642	ctcgacacggggcacatcg-ac-c-agctcgtgcgccatc
SPCCdxsdna	1630	ttagatgaggaactgattgtgc-c-gctggcgcgccagat
ECdxsdna	1612	cttgatgaagcggttaattc-tg-g-aaatggccgccagcc
NMdxsdna	1645	atagacgaagagttgattg-tc-c-gccttgcccgaagcc
HIdxsdna	1612	attgatattgaaatgatta-at-gtgcttgacaaa-actc
SSdxsdna	1615	gtcgaccccgctgctg-----c-ccccactcgcgcgcg
HPdxsdna	1600	ttagatccaaatttaagcg-cg-a-tcgttgccccttatc
STdxsdna	1851	acgaagtggcgggtga---cgatcgaggaa--ggcgc---g
CRdxsdna	1943	accctgtcatgatca---ccatcgaggag--ggctc---c
CJdxsdna	1635	taaaatttggtttat---ttttagtgaatgttaa---a
PAdxsdna	1664	acgaactgctgggtga---ccatcgaggaa--aacgccgtg
LEdxsdna	1910	atgaagtgctaataca---ctgtcgaagaa--ggatc---a
MTdxsdna	1652	acaagctgctcgtca---cgctagaggac--aacgg---g
RSdxs1dna	1667	atcacgaggcgctcattaccatcgaggag--ggcgc---c
RSdxs2dna	1679	acgcggcgctggtaa---cggtggagcag--ggggc---c
SPCCdxsdna	1668	cggcaaagtcg-tca---cctttgaggaa--ggctg---c
ECdxsdna	1649	atgaagcgtggtca---ccgtagaagaa--aacgc---c
NMdxsdna	1682	acgaccgcacgtta---cccttgaagaa--aacgcc--g
HIdxsdna	1649	acgattatttggtca---cattggaagaa--aatgc---a
SSdxsdna	1646	agcaccggctcgtcg---ccgtcgtggag--gac-----
HPdxsdna	1637	aaaagctctatgttt---ttagcgataat--tacaa---g

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STdxsdna	1883	atc--ggcggccccggt-gcgc-atgtgctgacg-----
CRdxsdna	1975	gtg--ggtggcttcgct-gcgc-acgtgatgcag-----
CJdxsdna	1669	att--ggcggatatagaaagttt-aattaataatt-----
PAdxsdna	1699	atg--ggcggcgccggc-tcg-----gcggtcggcgagt
LEdxsdna	1942	att--ggaggttttgga-tctc-atgttggttcag-----
MTdxsdna	1684	gtc--aacggtggggcg-gggt-cagcgggtg-----
RSdxs1dna	1702	atc--ggcggcttcggc-agcc-atgtggcgag-----
RSdxs2dna	1711	atg--ggcggcttcggc-gcct-atgtcatgcaactgt---
SPCCdxsdna	1699	cta--cccggcggtttt-ggct-ccgcgattatg-----
ECdxsdna	1681	att--atgggc-----g-gcgc-agg-----
NMdxsdna	1715	aacaggggcggcgcaggc-agcg-cggtgctggaa-----
HIdxsdna	1681	att--caagg---tgga-gcgggatctgctgttg-----
SSdxsdna	1675	aac--agccgggcccgc-gggg-tcggttcgggcg-----
HPdxsdna	1669	ctt--ggagg---ggt-g-----g-----
STdxsdna	1913	--ctc---gccagcgatac-cggcc--t---gatcgacg
CRdxsdna	2005	--ttc---ctcgactgga-gggcc--t---gctggacg
CJdxsdna	1700	--ttt---tacaaaaata-----t---gat----
PAdxsdna	1730	tcctc---gccagcga---gggcc--t-----
LEdxsdna	1972	--ttcatggccttagat---gggc--t---tcttgatg
MTdxsdna	1711	---tc---ggccgcgctgc-ggcgc--gcggagatcgacg
RSdxs1dna	1732	--ctt---ctggccgaggc-cgggg--t---cttcgacc
RSdxs2dna	1744	--ctc---gcca---attc-cggcg--g---cttcgacg
SPCCdxsdna	1729	--gag---tcc---ttgc-aggcccat---gac--tg
ECdxsdna	1698	----c---agcggcgtgaa-cgaag--t---gctgatgg
NMdxsdna	1747	--gt---gttggcgaaacacggca--t---ctgcaaac
HIdxsdna	1709	----c---ggaagtactaa-attca--t---caggaaaa
SSdxsdna	1705	--gtc---gccctggcgct-cgggg--a---cgccgatg
HPdxsdna	1682	--cta---gc--gcgattt-tagag--t---ttttga--
STdxsdna	1941	---ccggcctc---aagc-----tgcgccaccatgcg
CRdxsdna	2033	---gcgggctc---aagt-----tccggcccatgac
CJdxsdna	1717	---ttgcatgtaaaagt-----tgttagctttgaa
PAdxsdna	1749	----cgaagtc---ccgc-----tgctgcaactggg
LEdxsdna	2000	---gcaagttg---aagt-----ggagaccaatagt
MTdxsdna	1742	tgccctgcccgc---gatg-----t-----cggggt
RSdxs1dna	1760	---gcggtctc---cggt-----atcgctcgatggt
RSdxs2dna	1769	---ggggcctc---gcgc-----tccgggtcatgac
SPCCdxsdna	1753	---cagg---tt---ccgg-----tggtgccgatcgg
ECdxsdna	1724	---cccatcgt---aaaccagtaccggtgctgaacattgg
NMdxsdna	1775	---ccg---tc---ttgc-----t---tttggg
HIdxsdna	1735	---tcaaccgc---a-ct-----tttacaacttg-g
SSdxsdna	1733	---tcgacgta---ccgg-----tgcgccgcttcgg
HPdxsdna	1706	-----gcgaac-----

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STdxsdna	1966	cctgccggaca----tattccaggaccaggacaagcccga
CRdxsdna	2058	gctgccggacc----gctacatcgaccacggcgactaccg
CJdxsdna	1744	tatga-agaca----aatttattgaacatggaaa-----
PAdxsdna	1773	cctgcccgaact----actacgtcgaacacgccaagcccag
LEdxsdna	2025	tcttcctgata----gatacattgaccatggatctcctgt
MTdxsdna	1764	gccgcaggagt----tctacgagcacgcgtctcgaagcga
RSdxs1dna	1785	gctgcccgaca----cgttcatcgaccaaacagcgccga
RSdxs2dna	1794	gctgcccgacc----gcttcacatcgagcaggcgagccccga
SPCCdxsdna	1776	tggtcccgaatc----tcttggtggaacatgccagccctga
ECdxsdna	1758	cctgccggact----tctt-----tattccgc
NMdxsdna	1791	cgttgccgata----ccgtaaccggacacggcgatccgaa
HIdxsdna	1758	cttgccagattatatttattccacaagcgacaca---gcaa
SSdxsdna	1758	catccccgagc----agttcctcgcgacgccaggcgcg
HPdxsdna	1712	-----aaaa----tattttaaagcctgttaaaagcttt
STdxsdna	2002	g-----aagcagt-a-----tgacgaa-----g
CRdxsdna	2094	c-----gaccagc-t-----ggccatg-----g
CJdxsdna	1773	-----aacaagt-----gag-----g
PAdxsdna	1809	c-----gagatgc-t-----cgccgaa-----t
LEdxsdna	2061	t-----gatcagt-t-----ggcggaa-----g
MTdxsdna	1800	g-----gtgctg-----gccgat-----c
RSdxs1dna	1821	a-----gtgatgt-a-----tgccacc-----g
RSdxs2dna	1830	g-----gacatgt-a-----tgccgat-----g
SPCCdxsdna	1812	tgaatctaaccagg-agttgggcctgacg-----c
ECdxsdna	1781	a-----aggaactca-----ggaagaa-----a
NMdxsdna	1827	a-----aaacttt-t-----agacgat-----t
HIdxsdna	1795	g-----aagca-t-t-----ggcagat-----t
SSdxsdna	1794	t-----gaggtgc-t-----cgccgac-----a
HPdxsdna	1741	g-----aatcat-----tgatgaatttatcatg
STdxsdna	2019	cggg-gctgaacgccgcc-----aacatcgctc-----
CRdxsdna	2111	ccgg-cctcaccagccag-----cacatcgcc-----
CJdxsdna	1784	tgga-aaaaaatctagaa-----aaagatgtc-----
PAdxsdna	1826	gcgg-cctggatgccgcg-----ggcatcg-----
LEdxsdna	2078	ctgg-cctaaccacatct-----cacattgca-----
MTdxsdna	1814	tggg-gctta---ccgac-----caggacgt-----
RSdxs1dna	1838	ccgg-gctgaatgcggcc-----gacatagag-----
RSdxs2dna	1847	cggg-gctgcgggccgag-----gatatcgcg-----
SPCCdxsdna	1841	cgcg-tcagatggccgat-----cgcatcctc-----
ECdxsdna	1799	tgcgcgccgaactcggcc-----tcgat-----
NMdxsdna	1844	tggg-cttgagtgc-----c-----
HIdxsdna	1811	tagg-attggatacaaaa-----ggcattgaa-----
SSdxsdna	1811	tcgg-gctgaccccggtg-----gagatcgcc-----
HPdxsdna	1765	catg-g--gaacaccgcttttagtggaataatccttaggat

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STdxsdna	2045	--gacacggtgc---tgaagg-cgctc---cgctacaacg
CRdxsdna	2137	--tccaccgcgc---tcacca-ccctggggcgcgccaagg
CJdxsdna	1810	--aatagtttgt---tgacg-----aaag
PAdxsdna	1850	-----aaaagg-cagta---cg-----
LEdxsdna	2104	--gcaacagtat---ttaaca-tactt---gg-----aca
MTdxsdna	1836	--ggcccggcg-----gatc---accggctggg
RSdxs1dna	1864	--cggaaggcgc---tggaga-cgct-----
RSdxs2dna	1873	--gccaccgcgc---ggggcg-cgctcg---cccggggcg
SPCCdxsdna	1867	--gaaaagt-----tggaag-c-----cgtcaacg
ECdxsdna	1822	--gccgctggta---tggaag-c-----caaaatca
NMdxsdna	1858	--gaagcgtg-----gaacg-gcgtg---tgcg-----
HI dxsdna	1837	--gaaaaaattc---tcaa-----ctt---tattgcaa--
SSdxsdna	1837	--g-ggcggatc---gg--cg-cgagc---ctgcccgctgc
HPdxsdna	1802	tagacacagagagtttgactgacgcta---ttttaaaga
STdxsdna	2076	---ag---gccgag-----ctggccga-cgg---gg-t
CRdxsdna	2171	---ac---gccgccaagtctcactgt-cag---cgct
CJdxsdna	1829	---tt---ttaaaa-----ttttatca-----
PAdxsdna	1863	-----ccag-----cgtctcga-c-----
LEdxsdna	2130	---aa---ccagag-----a-ggctct-aga---gg-t
MTdxsdna	1859	---tc---gccgcg-----ctgggtac-cgg---gg-t
RSdxs1dna	1884	---g---ggggtg-----gaggtcct-cgc---cc-g
RSdxs2dna	1905	---cgtgatgccgct---ccggcaga-cggcaaagc-c
SPCCdxsdna	1890	---ga---ttggtg-----ctg---ctt-cgg---ct-t
ECdxsdna	1847	---ag---gcctgg-----ct-----
NMdxsdna	1881	---c---gcgtgg-----ctgtcggatcgg---ga-t
HI dxsdna	1862	-----aa-cao---gg-t
SSdxsdna	1865	---gg---gaggaa-----ccggccga-gga---gc-a
HPdxsdna	1839	ttag---gacaag---agagatga-----
STdxsdna	2098	gcgggcg--taa-----
CRdxsdna	2199	gcaagcg--taa-----
CJdxsdna	1845	-----t--taa-----
PAdxsdna	1876	-cggcag--tag-----
LEdxsdna	2151	catgaca--taa-----
MTdxsdna	1881	gtgtgcg--tccgacgcgattccagaacatctcgactaa
RSdxs1dna	1905	ccgcgcc--tga-----
RSdxs2dna	1935	gcgggcg--gtctga-----
SPCCdxsdna	1910	ga-----
ECdxsdna	1857	---ggca--taa-----
NMdxsdna	1903	gcggcaaattaa-----
HI dxsdna	1870	a-attta--taa-----
SSdxsdna	1887	gcccgca--tga-----
HPdxsdna	1853	-----

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STdxsp	182	-----
AAdxsp	1	-----
BSdxsp	1	-----
CRdxsp	1	mlrgavshgpa-----
CJdxsp	1	-----
PAdxsp	1	mpkt-----
LEdxsp	1	-----
MLdxsp	1	-----
MTdxsp	1	-----
RCdxsp	1	-----
RSdxslp	1	-----
RSdxs2p	1	mtn-----
SPCCdxsp	1	-----
SPdxsp	1	-----
TMdxsp	1	-----
ECdxsp	1	m-----
NMdxsp	1	-----
HIdxsp	1	m-----
PFdxsp	1	mifnyvffknfvpvlyilliiyinlngmnknqikteki
SSdxsp	1	-----
HPdxsp	1	-----
STdxsp	182	-----
AAdxsp	1	-----
BSdxsp	1	-----
CRdxsp	12	-----
CJdxsp	1	-----
PAdxsp	5	-----
LEdxsp	1	-----
MLdxsp	1	-----
MTdxsp	1	-----
RCdxsp	1	-----
RSdxslp	1	-----
RSdxs2p	4	-----
SPCCdxsp	1	-----
SPdxsp	1	-----
TMdxsp	1	-----
ECdxsp	2	-----
NMdxsp	1	-----
HIdxsp	2	-----
PFdxsp	41	yikklnrlsrknslecssknkiacldignddnrnttygyn
SSdxsp	1	-----
HPdxsp	1	-----

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STdxsp	182	-----
AAdxsp	1	-----
BSdxsp	1	-----
CRdxsp	12	-----
CJdxsp	1	-----
PAdxsp	5	-----
LEdxsp	1	-----
MLdxsp	1	-----
MTdxsp	1	-----
RCdxsp	1	-----
RSdxslp	1	-----
RSdxs2p	4	-----
SPCCdxsp	1	-----
SPdxsp	1	-----
TMdxsp	1	-----
ECdxsp	2	-----
NMdxsp	1	-----
HIdxsp	2	-----
PFdxsp	81	vnvknddinsllknnysnklymdkrkninnvistnkisgs
SSdxsp	1	-----
HPdxsp	1	-----
STdxsp	182	-----
AAdxsp	1	-----
BSdxsp	1	-----
CRdxsp	12	-----
CJdxsp	1	-----
PAdxsp	5	-----
LEdxsp	1	-----
MLdxsp	1	-----
MTdxsp	1	-----
RCdxsp	1	-----
RSdxslp	1	-----
RSdxs2p	4	-----
SPCCdxsp	1	-----
SPdxsp	1	-----
TMdxsp	1	-----
ECdxsp	2	-----
NMdxsp	1	-----
HIdxsp	2	-----
PFdxsp	121	isnicrnrqkenekrnrkqrcltqchtynmshqgdkland
SSdxsp	1	-----
HPdxsp	1	-----

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STdxsp	182	-----
AAdxsp	1	-----
BSdxsp	1	-----
CRdxsp	12	-----
CJdxsp	1	-----
PAdxsp	5	-----
LEdxsp	1	-----
MLdxsp	1	-----
MTdxsp	1	-----
RCdxsp	1	-----
RSdxs1p	1	-----
RSdxs2p	4	-----
SPCCdxsp	1	-----
SPdxsp	1	-----
TMdxsp	1	-----
ECdxsp	2	-----
NMdxsp	1	-----
HIdxsp	2	-----
PFdxsp	161	nnrnnkknfnllfynyfnlkrmknsllnkdnffyckekkl
SSdxsp	1	-----
HPdxsp	1	-----
STdxsp	182	-----
AAdxsp	1	-----
BSdxsp	1	-----
CRdxsp	12	-----
CJdxsp	1	-----
PAdxsp	5	-----
LEdxsp	1	-----
MLdxsp	1	-----
MTdxsp	1	-----
RCdxsp	1	-----
RSdxs1p	1	-----
RSdxs2p	4	-----
SPCCdxsp	1	-----
SPdxsp	1	-----
TMdxsp	1	-----
ECdxsp	2	-----
NMdxsp	1	-----
HIdxsp	2	-----
PFdxsp	201	sflhkaykkknctfqnyslkrksnrdshklfsgefddyt
SSdxsp	1	-----
HPdxsp	1	-----

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STdxsp	182	-----
AAdxsp	1	-----
BSdxsp	1	-----
CRdxsp	12	-----
CJdxsp	1	-----
PAdxsp	5	-----
LEdxsp	1	-----
MLdxsp	1	-----
MTdxsp	1	-----
RCdxsp	1	-----
RSdxslp	1	-----
RSdxs2p	4	-----
SPCCdxsp	1	-----
SPdxsp	1	-----
TMdxsp	1	-----
ECdxsp	2	-----
NMdxsp	1	-----
HIdxsp	2	-----
PFdxsp	241	nnalyesekkeyitlnnnnnknnnnknndnknndndynnn
SSdxsp	1	-----
HPdxsp	1	-----
STdxsp	182	-----
AAdxsp	1	-----
BSdxsp	1	-----
CRdxsp	12	-----
CJdxsp	1	-----
PAdxsp	5	-----
LEdxsp	1	-----
MLdxsp	1	-----
MTdxsp	1	-----
RCdxsp	1	-----
RSdxslp	1	-----
RSdxs2p	4	-----
SPCCdxsp	1	-----
SPdxsp	1	-----
TMdxsp	1	-----
ECdxsp	2	-----
NMdxsp	1	-----
HIdxsp	2	-----
PFdxsp	281	nscnnlgersnhydnyggdnnnpennndkydigkyfkqi
SSdxsp	1	-----
HPdxsp	1	-----

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STdxsp	182	-----m
AAdxsp	1	-----
BSdxsp	1	-----
CRdxsp	12	-----v
CJdxsp	1	-----
PAdxsp	5	-----l
LEdxsp	1	-----m
MLdxsp	1	-----
MTdxsp	1	-----
RCdxsp	1	-----m
RSdxslp	1	-----m
RSdxs2p	4	-----p
SPCCdxsp	1	-----
SPdxsp	1	-----
TMdxsp	1	-----
ECdxsp	2	-----s
NMdxsp	1	-----
HIdxsp	2	-----t
PFdxsp	321	ntfinideyktygdeiykeiyelyvernipeyyerkyfs
SSdxsp	1	-----
HPdxsp	1	-----m
STdxsp	185	a-----dl----
AAdxsp	1	-----ml----
BSdxsp	1	-----
CRdxsp	13	a-----draaag
CJdxsp	1	-----m----
PAdxsp	6	h-----ei----
LEdxsp	2	alcayafpgilnrtgvvsdsskatplfsgwihgtldlqflf
MLdxsp	1	-----
MTdxsp	1	-----
RCdxsp	2	s-----at----
RSdxslp	2	t-----dr----
RSdxs2p	5	t-----pr----
SPCCdxsp	1	-----
SPdxsp	1	-----
TMdxsp	1	-----
ECdxsp	3	f-----di----
NMdxsp	1	-----m----
HIdxsp	3	n-----nm----
PFdxsp	361	e-----di----
SSdxsp	1	-----
HPdxsp	2	i-----lq----

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STdxsp	194	-----
AAdxsp	3	-----
BSdxsp	1	-----
CRdxsp	20	parcaapvargvrssaaptrqrraeasvnapragpagsysg
CJdxsp	2	-----
PAdxsp	9	-----
LEdxsp	42	qhklthevkkrsrvvqaslsesgeyytqr-----
MLdxsp	1	-----
MTdxsp	1	-----
RCdxsp	5	-----
RSdxslp	5	-----
RSdxs2p	8	-----
SPCCdxsp	1	-----
SPdxsp	1	-----
TMdxsp	1	-----
ECdxsp	6	-----
NMdxsp	2	-----
HIdxsp	6	-----
PFdxsp	364	-----
SSdxsp	1	-----
HPdxsp	5	-----
STdxsp	194	-----p-k---t-----
AAdxsp	3	-----e-k---y-----
BSdxsp	1	-----m-----
CRdxsp	60	ewdklsveeidewrdvgp-k---t-----
CJdxsp	2	-----s-k-----
PAdxsp	9	-----p-rerpat-----
LEdxsp	71	-----p-p---t-----
MLdxsp	1	-----
MTdxsp	1	-----
RCdxsp	5	-----psr---t-----
RSdxslp	5	-----p-c---t-----
RSdxs2p	8	-----p-e---t-----
SPCCdxsp	1	-----
SPdxsp	1	-----
TMdxsp	1	-----
ECdxsp	6	-----a-k---y-----
NMdxsp	2	-----n-p---s-----
HIdxsp	6	-----n-n---y-----
PFdxsp	364	-----k-k---svlfdidkyndvefek
SSdxsp	1	-----m-----
HPdxsp	5	-----n-k---t-----

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STdxsp	203	-----plld
AAdxsp	6	-----eil k
BSdxsp	2	-----dll-
CRdxsp	80	-----plld
CJdxsp	4	-----k
PAdxsp	16	-----plld
LEdxsp	74	-----pild
MLdxsp	1	-----mle
MTdxsp	1	-----mlq
RCdxsp	9	-----phld
RSdxslp	8	-----ptld
RSdxs2p	11	-----plld
SPCCdxsp	1	-----mhls
SPdxsp	1	-----mhis
TMdxsp	1	-----mllld
ECdxsp	9	-----ptla
NMdxsp	5	-----plld
HIdxsp	9	-----plls
PFdxsp	382	aikeefinngvyinnidntyykkenilimkkilhyfpllk
SSdxsp	2	-----tile
HPdxsp	8	-----fdln
STdxsp	215	tvdtppqdlrklapaqlrqladelraetisavgstgghlgs
AAdxsp	10	dykgpfdiknydyetlqklaqevrdyiinvtskngghvgp
BSdxsp	5	siqdpfslknmsideleklsdeirqflitslsasgghigp
CRdxsp	84	tvnypvhlknfnneqlkqlckelrsdivhtvstgghlss
CJdxsp	5	fahtqeeleklslkelenlaasmrekiiqvsvskngghlss
PAdxsp	20	rasspaelrrlgeadletladelrqylltyvggtgghfga
LEdxsp	78	tvnypihmknslskelkqladelrsdtifnvsktgghlgs
MLdxsp	4	qirrpadiqlhlsqqqlrdlaaeirellvhkvaatgghlgp
MTdxsp	4	qirgpadiqlhlsqaqlrelaaeireflihkvaatgghlgp
RCdxsp	13	rvtgpadlkamsiadltalasevrreivevvsqtgghlgs
RSdxslp	12	rvtlvpdikgltdrelrsladelraetisavsvtgghlga
RSdxs2p	15	rvccpadmkalsdaelerladevrsevisvvaetgghlgs
SPCCdxsp	5	eithpnqlhglsvaqleqighqirekhlqtvaatgghlgp
SPdxsp	5	elthpnelkglsireleevsrqirekhlqtvatgghlgp
TMdxsp	5	-----eikrmsydelkrlaedirkrteevlknngghlas
ECdxsp	13	lvdstqelrllpkesslpklcdelrrylldsvsrsgghfas
NMdxsp	9	lidspqdlrrldkkqlprlagelrtfllesvgqtgghfas
HIdxsp	13	linspedlrllnkdqlpqlcqlrayllesvsqtsghlas
PFdxsp	422	linnpsdlkklkkqylpllaheikiflffivnitgghfss
SSdxsp	6	nirqpdlkalpeeqlhelseeirqflvhavtrtgghlgp
HPdxsp	12	----pndi-----aglelvcqtlrnrilevvsangghlss

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STdxsp	335	glgvveltvaihyvfntpddrliwdvghqcyphkiltgrr
AAdxsp	50	slgvveltiallrvfnpdpdvivwdighqgypwkiltgrk
BSdxsp	45	nlgvveltvalhkefnspkdkflwdvghqsyvhkiltgrg
CRdxsp	124	slgvveltvamhyvfntpedkiiwdvghqayghkiltgrr
CJdxsp	45	nlgavelsiamhlvfdakkdpfifdvshqsythkllsgke
PAdxsp	60	glgvveltialhyvfntpddrlvwdvghqayphkilterr
LEdxsp	118	slgvveltvalhyvfnapqdrilwdvghqsyphkiltgrr
MLdxsp	44	nlgvveltialhrvfdsphdpiifdtghqayvhkmltgrc
MTdxsp	44	nlgvveltialhrvfdsphdpiifdtghqayvhkmltgrs
RCdxsp	53	slgvveltvalhavfnspgdkliwdvghqcyphkiltgrr
RSdxslp	52	glgvveltvalhaifdaprdkiiwdvghqcyphkiltgrr
RSdxs2p	55	slgvveltvalhavfntptdklvwdvghqcyphkiltgrr
SPCCdxsp	45	glgvveltialyqtlldldrdkvwdvghqayphkiltgry
SPdxsp	45	glgvveltvalystldldkdrviwdvghqayphkmltgry
TMdxsp	39	nlgtieltlalyrvfdpredaiiwdtghqaythkiltgrd
ECdxsp	53	glgtveltvalhyvyntpfqdliwdvghqayphkiltgrr
NMdxsp	49	nlgaveltvalhyvyntpedklvwdvghqsyphkiltgrk
HIdxsp	53	glgtveltvalhyvyktpfdqliwdvghqayphkiltgrr
PFdxsp	462	vlssleiqllllyifnqpydnviydighqayvhkiltgrk
SSdxsp	46	nlgvveltialhrvfespvdrilwdtghqsyvhkiltgrq
HPdxsp	43	slgavelivgmhalfdcqknpfifdtshqayahkiltgrf
STdxsp	455	drirtirqggglsgftkrseseydpfgaahsstsisaalg
AAdxsp	90	eqfptlrqykgisgflrreesiydafgaghsstsisaalg
BSdxsp	85	kefatlrqykglgcfpkrsesehdvwetghsstsislsgamg
CRdxsp	164	kgmtirqtnglsgftkrdeseydpfgaghsstsisaalg
CJdxsp	85	eifdtlrqinglsgytkpsegdy--fvaghsstsislavg
PAdxsp	100	elmgtlrqknglaafprraeseydtfgvghsstsisaalg
LEdxsp	158	dkmstlrqtdglagftkrseseydcfgtghssttisaglg
MLdxsp	84	qdfdslrkkaglsygypsraesehdwvesshastalsyadg
MTdxsp	84	qdfatlrkkaglsygypsraesehdwvesshasaalsyadg
RCdxsp	93	srmltlrqaggisgfpkrsephdafgaghsstsisaalg
RSdxslp	92	drirtlrqggglsgftkrsepydcfgaghsstsisaavg
RSdxs2p	95	eqmrtlrqkgglsgftkrseaydpfgaahsstsisaalg
SPCCdxsp	85	hnfhrtlqkdgiagypkrtenrfdhfgaghasstsisaglg
SPdxsp	85	hdfhtlrqkdgvagylkrsestrfdhfgaghasstsisaglg
TMdxsp	79	dlfhrtirtfgglsgfvtrrespldwfgtgghagtsiaaglg
ECdxsp	93	dkigtirqkgglhpfpwrgeseydvlsvghsstsisaaglg
NMdxsp	89	nqmhtmrqygglagfpkrceseydafgvghsstsisaaglg
HIdxsp	93	eqmstirqkdgihpfpwreeseefdlsvghsstsisaaglg
PFdxsp	502	llflslrnkkgisgflnifesiydkfgaghsstsisaigq
SSdxsp	86	d-fsklrgkgglsgypsreesehdvienshastalgwadg
HPdxsp	83	esfstlrqfkgglsgftkpsesaydyfiaghsstsvsigvg

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STdxsp	575	fa-----
AAdxsp	130	fr-----
BSdxsp	125	ma-----
CRdxsp	204	ma-----
CJdxsp	123	ac-----
PAdxsp	140	ma-----
LEdxsp	198	ma-----
MLdxsp	124	la-----
MTdxsp	124	la-----
RCdxsp	133	fa-----
RSdxslp	132	fa-----
RSdxs2p	135	fa-----
SPCCdxsp	125	ma-----
SPdxsp	125	ma-----
TMdxsp	119	fe-----
ECdxsp	133	ia-----
NMdxsp	129	ma-----
HIdxsp	133	ia-----
PFdxsp	542	yyeaewqvknkekyngdieisdnanvtnnerifqkgihn
SSdxsp	125	la-----
HPdxsp	123	va-----
STdxsp	581	---iankln-----eapgk-a-----
AAdxsp	132	---igkdlkg-----ekedy-v-----
BSdxsp	127	---aardik-----gtdey-i-----
CRdxsp	206	---vgrdvk-----gkks-v-----
CJdxsp	125	---kaialk-----gekri-p-----
PAdxsp	142	---iaarlq-----gkerk-s-----
LEdxsp	200	---vgrdlk-----grnnn-v-----
MLdxsp	126	---kafela-----gnrnrhv-----
MTdxsp	126	---kafelt-----ghrnrhv-----
RCdxsp	135	---vgrelg-----qpvgd-t-----
RSdxslp	134	---aaremng-----gdtgd-a-----
RSdxs2p	137	---mgrelg-----qpvgd-t-----
SPCCdxsp	127	---lardaq-----gedyr-c-----
SPdxsp	127	---lardak-----gedfk-v-----
TMdxsp	121	---kafell-----gekrh-v-----
ECdxsp	135	---vaaeke-----gknrr-t-----
NMdxsp	131	---aadkql-----gsdrr-s-----
HIdxsp	135	---vaaere-----nagr-k-t-----
PFdxsp	582	dnninnnnnnnyinpsdvvgr-entnvpnrndnhnvdk
SSdxsp	127	---karrvq-----gekgh-v-----
HPdxsp	125	---kafcl-k-----qalgm-p-----

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STdxsp	617	--iavigdgamsagmayeamnna-eaagmr-lvvilndnd
AAdxsp	145	--iavigdgaltagmayealnaghirpdr-fivilndne
BSdxsp	139	--ipiigdgaltggmalealnhhi-gdekdd-mivilndne
CRdxsp	218	--iavigdgaitggmayeamnha-gfldkn-mivilndnq
CJdxsp	137	--valigdgalsagmayealnel-gdskfp-cvillndne
PAdxsp	154	--vavigdgaltagmafealnhha-sevdad-mlvilndnd
LEdxsp	212	--iavigdgamttagqayeamnna-gyldsd-mivilndnr
MLdxsp	139	--vavvgdgaltggmcwealnni-aatprp-vvivvndng
MTdxsp	139	--vavvgdgaltggmcwealnni-aasrrp-viivvndng
RCdxsp	147	--iaiiigdgisitagmayealnhha-ghlksr-mfvilndnd
RSdxslp	146	--vavigdgmsagmafealnhg-ghlknr-vivilndne
RSdxs2p	149	--iavigdgisitagmayealnhha-ghlnkr-lfvilndnd
SPCCdxsp	139	--vavigdgsltggmaleainhaghlpktr-llvvilndnd
SPdxsp	139	--vsiigdgaltggmaleainhaghlphtr-lmvilndne
TMdxsp	133	--vvvigdgaltsgmalealnql-knlnsk-mkiilndng
ECdxsp	147	--vcvigdgaitagmafeamnhha-gdirpd-mlvilndne
NMdxsp	143	--vaiigdgamttagqafealnca-gdmvd-llvvilndne
HIdxsp	147	--vcvigdgaitagmafealnhha-galhtd-mlvilndne
PFdxsp	621	vhiaiiigdgglttggmalealnyi-sflnsk-iliinyndng
SSdxsp	139	--vaviggraltggmawealnni-aaakdqpliivvndne
HPdxsp	137	--iallgdgsisagifyealnel-gdrkyp-mimilndne
STdxsp	725	msiap-----pvgglsayl--arlissseyl--gl
AAdxsp	182	msisp-----nvgaistyl--nriisghfvq--et
BSdxsp	175	msiap-----nvgaihsml--grlrtagkyq--wv
CRdxsp	254	qvsllptqynnknqd-pvgalssal--arlqanrplr--el
CJdxsp	173	msisk-----pigaiskyl--sqamatqfyq--sf
PAdxsp	190	msish-----nvgglsnyl--akilssrtyt--sm
LEdxsp	248	qvsllptatldgpva-pvgalssal--srlqsnrplr--el
MLdxsp	175	rsyap-----tiggvadhl--atrlrlqpaye--rl
MTdxsp	175	rsyap-----tiggvadhl--atrlrlqpay----
RCdxsp	183	msiap-----pvgalqhyl--ntiarqapfa--al
RSdxslp	182	msiap-----pvgalssyl--srlyagapfq--df
RSdxs2p	185	msiap-----pvgalaryl--vnlsskapfa--tl
SPCCdxsp	176	msisp-----nvgalstryl--nk-irvsepm--ql
SPdxsp	176	msisp-----nvgaistrylnkvrllsspmqfltdnl
TMdxsp	169	msisp-----nvgglayhl--sklrtspiyl--kg
ECdxsp	183	msise-----nvgalnnhl--aqllsgklyt--sl
NMdxsp	179	msisp-----nvgalpkyl--asnvvrdmh--gl
HIdxsp	183	msise-----nvgalnnhl--arifsgslyt--tl
PFdxsp	659	qvsllptnavsisgnrpigsisdhl--hyfvsnie-----
SSdxsp	176	rsyap-----tigglanhl--atlrtdtdgye--kv
HPdxsp	173	msist-----pigalskal--sqlmkgpfyq--sf

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STdxsp	803	relakrf---trk--lsr-----rltaa---a-gkaeef
AAdxsp	208	rqkiknf---lqh--fge-----tplri---m-klteef
BSdxsp	201	kdeleyl---fkk--ipavgg---klaat---a-ervkds
CRdxsp	289	reiakgv---tkq--lpd-----vvqka---t-akidey
CJdxsp	199	kkriakm---ldi--lpd-----satym---a-krfees
PAdxsp	216	regsk-----k--vls-----rlpgaweia-rrteey
LEdxsp	283	revakgv---tkq--igg-----pmhel---a-akvdey
MLdxsp	201	lekg-----rd--alh-----slpli---g-qiayrf
MTdxsp	198	-eqalet---grd--lvr-----avplv---g-glwfrf
RCdxsp	209	kaaaegi---emh--lpg-----pvr dg---a-rrarqm
RSdxslp	208	kaaakga---lgl--lpe-----pfqeg---a-rrakem
RSdxs2p	211	raaadgl---eas--lpg-----plrdg---a-rrarql
SPCCdxsp	201	--ltdgl---tgg--mqqipfvvggaitgg---f-epvkeg
SPdxsp	206	eeqikhl---pf---vgd-----sltpe---m-ervkeg
TMdxsp	195	kkvlkkv---lekteigf-----eveee---m-kylrds
ECdxsp	209	reggkkv---fsg--vp-----pikel---l-krteeh
NMdxsp	204	lstvkaq---tgk--vld-----kipgamefa-qkvehk
HI dxsp	209	rdgskki---ldk--vp-----piknf---m-kkteeh
PFdxsp	691	---anag---dnk--lsk-----n-----
SSdxsp	202	lawgkdvlrtpi---vgh-----plyea---lhgakkgf
HPdxsp	199	rskvkki---lst--lpe-----svnyl---a-srfees
STdxsp	878	argm--atg-----g-----tlfeelgfyyvgpidg
AAdxsp	233	lkg1--isp-----g-----vifeelgfnyigpidg
BSdxsp	229	lkym--lvs-----g-----mffeelgftyigpvdg
CRdxsp	314	argmisgtg-----s-----tlfeelglyyigpvdg
CJdxsp	224	fk-l--itp-----g-----llfeelgleyigpidg
PAdxsp	240	akgm--lvp-----g-----tlfeelgwnyigpidg
LEdxsp	308	argmisgsg-----s-----tlfeelglyyigpvdg
MLdxsp	222	mhsv--kagikdslspq-----llftdlglkyvgpvdg
MTdxsp	222	lhsv--kagikdslspq-----llftdlglkyvgpvdg
RCdxsp	234	vtam--pgg-----a-----tlfeelgfdyigpvdg
RSdxslp	233	lksv--tv g-----g-----tlfeelgfsyvgpidg
RSdxs2p	236	vtgm--pgg-----g-----tlfeelgftyvgpidg
SPCCdxsp	230	mkrl--syski-----g-----avfeelgftymgpvdg
SPdxsp	230	mkrl--vvpkv-----g-----avieelgfkyfgpidg
TMdxsp	222	lkgm--iqg-----t-----nffeslgkyfgpfdg
ECdxsp	233	ikgm--vvp-----g-----tlfeelgfnyigpvdg
NMdxsp	232	iktl--aee-----aehakqslslfenfgfrytgpvdg
HI dxsp	233	mkgvmfspe-----s-----tlfeelgfnyigpvdg
PFdxsp	702	-----ake-----n-----nifenlnydyigvng
SSdxsp	231	kdaf--apq-----g-----mfedlgkyvgpidg
HPdxsp	224	fk-l--itp-----g-----vffeelginyigping

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STdxsp	950	hnlehlipvlenvrdse-q-gpilihvvtkkkgkyapaea
AAdxsp	257	hdikaledtlennvkd--k-gpvllhvvtkkkgkykpaea
BSdxsp	253	hsyheliienlqyakkt--k-gpvllhvvtkkkgkykpae
CRdxsp	340	hnlddliavlsevrsae-tvgpvlhvvttekgrgylpaet
CJdxsp	247	hnlgeiisalkqak-am-q-kpcvihaqtikkgkyalaeg
PAdxsp	264	hdlptlvatlrmrdm--k-gpqflhvvtkkkgkfapael
LEdxsp	334	hniddliailkevrtk-ttgpvlhvvttekgrgypyaer
MLdxsp	253	hd-ehavevalrkargf-g-gpvivhvvttrkgmgypaea
MTdxsp	253	hd-eravevalrsarrf-g-apvivhvvttrkgmgypaea
RCdxsp	258	hdmaelvettlrvtara-s-gpvlihvtcttkkgkyapae
RSdxs1p	257	hdldqllpvlrtvkqra-h-apvlihvitkkgrgyapaea
RSdxs2p	260	hdmeallqtlraarart-t-gpvlihvttkkgkyapae
SPCCdxsp	256	hnleeliatfreah-kh-t-gpvlhvvttkkgkyapae
SPdxsp	256	hslqelidtfkqa-ekv-p-gpvfwhvsttkkgkydlaek
TMdxsp	246	hniellekvfkrirdyd-y-ssv-vhvvttkkgkftaaee
ECdxsp	257	hdvlglttlknmrld--k-gpqflhimtkkggyepaek
NMdxsp	263	hnvenlvdvledlr-gr-k-gpqllhvvttkkgkyklaen
HIdxsp	259	hnidelvatltmrnl--k-gpqflhikttkkgkyapae
PFdxsp	722	nnteelfkvlnnikenklk-ratvlhvrtkksndfinsks
SSdxsp	254	hdigavesalrrak-rf-h-gpvlvhcltvtkggyepala
HPdxsp	247	hdlsaiietlklakelk-e--pvlihaqtlkgkykiaeg
STdxsp	1064	-aadkyhgvqk-----fd--vitg-aqaka-----pp---
AAdxsp	294	-npvkwhgvap-----yk--vesg-eiik-----ks---
BSdxsp	290	dtigtwhgtgp-----yk--intg-dfvkp-----ka---
CRdxsp	379	-aqdkmhgvvk-----fd--prtq-kqvqa-----kt---
CJdxsp	284	-khakwhgvga-----fd--idsg-esvkk-----sd---
PAdxsp	301	-dpigyhaik-----le--apgs-apkkt--------
LEdxsp	373	-aadkyhgvak-----fd--patg-kqfka-----sa---
MLdxsp	290	dqaeqmhtcgv-----md--pttg-qptki--------
MTdxsp	290	dqaeqmhtcgv-----id--patg-qatk--------
RCdxsp	296	-aedklhgvsk-----fd--ietg-kqkks-----ip---
RSdxs1p	295	-ardrghatnk-----fn--vltg-aqvkp-----vs---
RSdxs2p	298	-apdkyhgvnk-----fd--pvtg-eqkks-----va---
SPCCdxsp	293	-dqvgyhaqnp-----fd--latgkakpas-----kp---
SPdxsp	293	-dqvgyhaqsp-----fn--lstgkaypss-----kp---
TMdxsp	283	-nptkyh-----sas-----ps---
ECdxsp	294	-dpitfhavpk-----fd--pssg-clpks-----sg---
NMdxsp	300	-dpvkyhavan-----lp--kesa-aqmpsekepka---
HIdxsp	296	-dpigfhgvpk-----fd--pisg-elpk-----nn---
PFdxsp	761	-pisilhsikkneifpfdttilng-nihke-----nkies
SSdxsp	291	heedhfhtvgv-----md--plt--cepls-----pt---
HPdxsp	284	-ryekwhgvqp-----fd--ldtg-lskks-----ks---

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STdxsp	1133	-----gpp---ay
AAdxsp	316	-----spp---tw
BSdxsp	314	-----aap---sw
CRdxsp	402	-----kam---sy
CJdxsp	307	-----tkk---sa
PAdxsp	322	-----gpp---ky
LEdxsp	396	-----ktq---sy
MLdxsp	312	-----aap---dw
MTdxsp	312	-----agp---gw
RCdxsp	319	-----nap---ny
RSdxslp	318	-----nap---sy
RSdxs2p	321	-----nap---ny
SPCCdxsp	317	-----kpp---sy
SPdxsp	317	-----kpp---sy
TMdxsp	294	-----gkpkmlsy
ECdxsp	317	-----glp---sy
NMdxsp	328	-----akp---ty
HIdxsp	318	-----skp---ty
PFdxsp	794	eknvssstkydvnnknnkndnseiikyedmfske---tf
SSdxsp	314	-----dgp---sw
HPdxsp	307	-----ail---sp
STdxsp	1148	tkvfadallaeaerdasvcaitaampsgtgldkfqatfpd
AAdxsp	321	tsvfgkalvelaerdekivaitpamregsglvefakrfpd
BSdxsp	319	sglvsgtvqrmaredgrivaitpampvgsklegfakrfpd
CRdxsp	407	tnyfadaltaeaerdsrivavhaamaggtglyrfekkkfpd
CJdxsp	312	teifsknlldlaskyenivgvtaampsgtgldklikeypn
PAdxsp	327	ssvfgqwlcdmaaqaarllgitpamkegsdlvafserype
LEdxsp	401	ttyfaealiaeaeadkdivaihaamgggtgmnlfhrrfpt
MLdxsp	317	taifsdaligyamkrrdivaitaampgptgltafgqcfpd
MTdxsp	317	tatfsdaligyagkrrdivaitaampgptgltafgqrfpd
RCdxsp	324	tavfgerlteeaardqaivavtaampgtgtgldimqkrfpr
RSdxslp	323	tkvfaqslikeaevdericavtaampdgtglnlfgerfpk
RSdxs2p	326	tkvfgstlteeaardprivaitaampsgtgvdimqkrfpr
SPCCdxsp	322	skvfgqtlttlaksdrrivgitaamatgtgldilqkalpk
SPdxsp	322	skvfahltltlakenpnivgitaamatgtgldklqaklpk
TMdxsp	302	sellghtlsrvaredkkivaitaamadgtglsifqkehpd
ECdxsp	322	skifgdwlcetaakdnklmaitpamregsgmvefsrkfpd
NMdxsp	333	tqvfgkwlcdradaadsrlvaitpamregsglvefeqrfpd
HIdxsp	323	skifgdwlcemaekdakiigitpamregsgmvefsqrfpk
PFdxsp	831	tdiytnemlkylkkdrniiflspamlggsglvkiserypn
SSdxsp	319	tsvfgdeivrigaeredivaitaamlhvpglarfadrfpd
HPdxsp	312	teaysntllelakkdekivgvtaampsgtgldklidaypl

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STdxsp	1268	rtfdvaiaeqhavgtaaglaa-qgmrpfcaiystflqray
AAdxsp	361	rffdvgiaeqhactfaaglaa-eglrpvaaystflqray
BSdxsp	359	rmfdvgiaeqhaatmaaamam-qgmkpflaiystflqray
CRdxsp	447	rtfdvgiaeqhavgtaaglac-eglvpfctiystfmqrgy
CJdxsp	352	rfwdvaiaeqhavgtsmaamak-egfkpfiaiystflqray
PAdxsp	367	ryfdvaiaeqhavgtaagmac-egmkpvvaiystflqray
LEdxsp	441	rcfdvgiaeqhavgtaaglac-egikpfcaiysfmgay
MLdxsp	357	rlfdvgiaeqhamtsaaglam-grmhpvvaiystflnraf
MTdxsp	357	rlfdvgiaeqhamtsaaglam-gglhpvvaiystflnraf
RCdxsp	364	rvfdvgiaeqhavgtaagmaa-aglkpfalysfvmqrgy
RSdxslp	363	rtfdvgiaeqhavgtsaalaa-qgmrpfcaiystflqrgy
RSdxs2p	366	rvfdvgiaeqhavgtaaglag-agmkpfcaiysfvlqrgy
SPCCdxsp	362	qyidvgiaeqhavglaagmac-dgmrpvvaiystflqraf
SPdxsp	362	qyvdvgiaeqhavgtaagmac-egirpvvaiystflqrgy
TMdxsp	342	rffdlgiteqtcvtfgaalgl-hgmkpvvaiystflqray
ECdxsp	362	ryfdvaiaeqhavgtaaglai-ggypkivaiystflqray
NMdxsp	373	ryfdvgiaeqhavgtaaglac-egmkpvvaiystflqray
HIdxsp	363	qyfdvaiaeqhavgtaaglai-ggypkvvaiystflqray
PFdxsp	871	nvydvgiaeqhsvtfaaamamnkklkiqlciystflqray
SSdxsp	359	rvwdvgiaeqhaavsaaglat-gglhpvvavaytflnraf
HPdxsp	352	rffdvaiiaeqhaltsssamak-egfkpfvsiystflqray
STdxsp	1385	dqvvhdvaiqnlpvrfaidraglvgadgathagsfdvtyl
AAdxsp	400	dqvihdvalqnlpvtfaidraglvgddgpthhgvdlsyl
BSdxsp	398	dqvvhdicrqnanvfigidraglvgadgethqgvfdiafm
CRdxsp	486	dqivhdvslqklpvrfaidraglvgadgthcgafdvtfm
CJdxsp	391	dqvihdcaimnlvfvfamidragivgedgthqgvfdlsfl
PAdxsp	406	dqlihdvavqhlldvlfaidraglvgedgpthagsfdlsyl
LEdxsp	480	dqvvhdvdlqklpvrfaidraglvgadgpthcgafdvtyl
MLdxsp	396	dqimmdvalhklpvtmvidragitgsdgpsnngmwdlsml
MTdxsp	396	dqimmdvalhklpvtmvidragitgsdgashngmwdlsml
RCdxsp	403	dqlvhdvalqnlpvrfaidraglvgadgathagafdvsm
RSdxslp	402	dqivhdvaidqrlpvrfaidraglvgadgathagsfdvaf
RSdxs2p	405	dqiahdvalqnlpvrfaidraglvgadgathagafdvvgfi
SPCCdxsp	401	dqvihdvciqklpvffcldragivgadgpthqgmydiayl
SPdxsp	401	dqiivhdvciqklpvffcldragivgadgpthqgmydiayl
TMdxsp	381	dqiivhdvalqnapvlfaidrsgvvgedgpthhglfdinyl
ECdxsp	401	dqvlhdvaidqklpvlfaidragivgadgpthqgafdlsl
NMdxsp	412	dqlvhdialqnlpvlfaidragivgadgpthaglydlsfl
HIdxsp	402	dqlihdvaidqnlpvlfaidragivgadgathqgafdisfm
PFdxsp	911	dqiivhdlnlqniplkviigrsglvgedgathqgiydlsl
SSdxsp	398	dqlimmdvalhrcgvtfvldragvtgvdgashngmwdmsvl
HPdxsp	391	dsivhdacisslpiklaidragivgedgthqglldvsl

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STdxsp	1505	aslpnfvmvmaadevelvbmthtaamhdsg-pialryprg
AAdxsp	440	rcvpnmvvcapkdeqelrdllytg-iysgk-pfalryprg
BSdxsp	438	rhlpnmvlmmpkdenegqhmvtalsydeg-piamrfprg
CRdxsp	526	aslpnmitmapsneaelinmvatcaaidda-pscfrfprg
CJdxsp	431	aplpnftllaprdeqmmqnimeyaylh-qg-pialryprg
PAdxsp	446	rcipgmlvmtpsdedelrkllttgylfd-g-paavryprg
LEdxsp	520	aclpnmvmapsdeaelfhmvatataaaddr-pscfryprg
MLdxsp	436	givpgmrvaaprdairlreelgealdvddg-ptairfpkg
MTdxsp	436	givpgirvaaprdatrlreelgealdvddg-ptalrfpkg
RCdxsp	443	anlpnftvmaadeaelchmvvtaaaahdsg-pialryprg
RSdxslp	442	snlpgivvmaadeaelvbmvatataahdeg-piafryprg
RSdxs2p	445	tslpnmtvmaadeaelihmiatavafgeg-piafrfprg
SPCCdxsp	441	rlipnmvlmapkdeaqlrmlvtgieyd-g-piamrfprg
SPdxsp	441	rcipnlvlmapkdeaqlqmlvtgvnytg-g-aiafryprg
TMdxsp	421	lpvpnmkiispsspeefvnslytlvkhldg-pvairypke
ECdxsp	441	rcipemvimtpsdeneqrmltytgyhyndg-psavryprg
NMdxsp	452	rcipnmivaapsdenecrlllstcyqada--paavryprg
HIdxsp	442	rcipnmiimtpsdeneqrmltytg--yqcgkpaavryprg
PFdxsp	951	gtlnnayiispsnqvdlkralrfayldkdh-svyiriprm
SSdxsp	438	qvvpglriaaprdadhvraqreavavdda-ptlirfpk-
HPdxsp	431	rsipnmvifaprdnetlknavrxfanhdss-pcafryprg
STdxsp	1622	n-----gvglalpk-----vp-erle-----
AAdxsp	478	a-----aygvpteg-----f--kkie-----
BSdxsp	477	n-----glgvkmd-----ql-ktip-----
CRdxsp	565	n-----glgldlaaygiskdlkgvp---le-----
CJdxsp	469	s-----fi-ldkef-----np-ceik-----
PAdxsp	484	s-----gpnhpdp-----dl-qpve-----
LEdxsp	559	n-----gigvelpagnkg-----ip---le-----
MLdxsp	475	d-----vcedipa-----lk-rrsg-----
MTdxsp	475	d-----vgedisa-----le-rrgg-----
RCdxsp	482	e-----grgvempe-----rg-evle-----
RSdxslp	481	d-----gvgvvvpv-----kg-vplq-----
RSdxs2p	484	e-----gvgvempe-----rg-tvle-----
SPCCdxsp	479	n-----gigvplpe-----egweslp-----
SPdxsp	480	n-----gigvplme-----egweple-----
TMdxsp	460	s-----fygevesl-----le-nmke-----
ECdxsp	480	n-----avgveltp-----l--eklp-----
NMdxsp	490	t-----gtgvpsd-----gm-etve-----
HIdxsp	480	n-----avgvkltp-----l--emlp-----
PFdxsp	990	nilsdkymkgylnihmkn-----es-knidvnvdin
SSdxsp	476	e-----svg---pr-----ip-aldr-----
HPdxsp	470	s-----falkegvf-----ep-sgfv-----

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STdxsp	1667	-----igkg-r-vvr-----
AAdxsp	492	-----igtw-e-ell-----
BSdxsp	492	-----igtw-e-vlr-----
CRdxsp	587	-----vgkg-v-vrr-----
CJdxsp	483	-----lgka-qwlvk-----
PAdxsp	499	-----igkg-v-vrr-----
LEdxsp	576	-----vgkg-r-ili-----
MLdxsp	489	-----vdl-a-vpa-----
MTdxsp	489	-----vdl-a-apa-----
RCdxsp	497	-----igkg-r-vmt-----
RSdxslp	496	-----igr-g-r-vvs-----
RSdxs2p	499	-----pgr-g-r-vvr-----
SPCCdxsp	495	-----igka-e-qlr-----
SPdxsp	496	-----igka-e-ilr-----
TMdxsp	475	-----idl-g-wk-ilk-----
ECdxsp	494	-----igkg-i-vkr-----
NMdxsp	505	-----igkg-i-irr-----
HIdxsp	494	-----igks-r-lir-----
PFdxsp	1020	ddvdkyseeymdddnfiksfiks-r-iikmdnennntne
SSdxsp	488	-----vggl-d-vlhrd-----
HPdxsp	485	-----lgqs-e-llk-----
STdxsp	1691	-----eg--kk--vails-l-g-tr-laealkaadtlea
AAdxsp	500	-----eg--ed--cvilavgyppyqalraaeklyk
BSdxsp	500	-----pg--nd--aviltfgttiemaieaaeelqk
CRdxsp	595	-----qg--kd--vclvaygssvnealaaadmler
CJdxsp	492	-----nn--se--iaflgygqgvakawqvlralqe
PAdxsp	507	-----rg--gr--vallvfgvqlaeamkvaeslda
LEdxsp	584	-----eg--er--vallgygsavqncldaaivles
MLdxsp	497	-----tglaqd--vllvgvgvfasmalavakrlhn
MTdxsp	497	-----dg--lnhdvllvaigafapmalavakrlhn
RCdxsp	505	-----eg--te--vailsfgahlgaalkaaemlea
RSdxslp	504	-----eg--tr--iallsfgtrlaevqvaaealaa
RSdxs2p	507	-----eg--td--vailsfgahlhealqaakllea
SPCCdxsp	503	-----qg--dd--llmlaygsmvypalqtaellne
SPdxsp	504	-----sg--dd--vlllgygsmvypalqtaellhe
TMdxsp	484	-----rg--re--aaiiatgtlnevlkip----
ECdxsp	502	-----rg--ek--lailnfgtlmpeaakvaeslne
NMdxsp	513	-----eg--ek--tafiafgsmvapalavagklne
HIdxsp	502	-----kg--qk--iailnfgtllpsalelseklne
PFdxsp	1058	hyssrgdtqtck--kk--vcifnmgsmlfnvinaikeiek
SSdxsp	498	-----er--pe--vllvavgvmaqvclqtaellra
HPdxsp	493	-----ke--ge--illigyngvgrahlvqlalke

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STdxsp	1769	k-----glsttvadlrfakpldedlirrl--tthevavt
AAdxsp	526	e-----girvgvvnarfvpkpmdekmlrdla--nrydtfit
BSdxsp	526	e-----glsvrvvnarfikpidekmmksil--keglpilt
CRdxsp	621	d-----gvsttvidarfckpldtklirsaa--kehpvmit
CJdxsp	518	m-----nnnanlidlifakpldeellcela--kkskiwfi
PAdxsp	533	-----tvvdmrfvfkpldealvrela--gshellvt
LEdxsp	610	r-----glqvtvadarfckpldhalirsla--kshevlit
MLdxsp	525	q-----gigvtvidprwvlpvcdgvl--ela--hthklivt
MTdxsp	525	q-----gigvtvidprwvlpv--sdgvrela--vqhklivt
RCdxsp	531	e-----gvsttvadarfcrpldtldlirli--eghaalit
RSdxslp	530	r-----gisptvadarfakpldrdlilqla--ahhealit
RSdxs2p	533	e-----gsvtvadarfsrpldtghidqlv--rhhaalvt
SPCCdxsp	529	h-----gisatvinarfakpldeelvpla--rqigkvvt
SPdxsp	530	h-----gieatvvvnarfvpkpldtelilpla--erigkvvt
TMdxsp	505	-----ldvtvvnaltvkpldtavlkeia--rdhdliit
ECdxsp	528	-----tlvdmrfvfkpldealilema--ashealvt
NMdxsp	539	-----tvadmrfvfkpideelivrla--rshdrivt
HIdxsp	528	-----tvvdmrfvfkpidieminvla--qthdylvt
PFdxsp	1094	eqyishnysfsivdmiflnpldknmidhvikqnkhqylit
SSdxsp	524	r-----gigctvvdprwvkpv--dpvlppla--aehrlvav
HPdxsp	519	k-----niecaldlrlfkpldpnlisaiva--pyqklyvf
STdxsp	1868	ieega-i-ggpgahv----ltlasdtglida-glklrtmr
AAdxsp	559	vednt-vvggfgsgv----leffaregimk---rvnlg
BSdxsp	559	ieeav-leggfgssi----lefahdgg--ey-htpidrmg
CRdxsp	654	ieegs-v-ggfaahv----mqflaleglldg-glklfrpmt
CJdxsp	551	fsenvki-ggiesli----nnflqk---ydl-hvkvvse
PAdxsp	561	ieena-vmggagsav----geflasegl---evpllqlg
LEdxsp	643	veegs-i-ggfgshv----vqfmaldglldg-klkwrpiv
MLdxsp	557	ledng-vnggvgaav----stalrq---vei-dtpcrdv
MTdxsp	557	ledng-v-nggagsa----vsaalrraeid---vpcrdvg
RCdxsp	564	leqga-m-ggfgamv----lhylartgglek-grairtmt
RSdxslp	563	ieega-i-ggfgshv----aqllaeagvfdg-gfryrsmv
RSdxs2p	566	veqga-m-ggfgayv----mhclansggfdg-glalrvmt
SPCCdxsp	562	feegc-l---pggfg----saimeslqahdl-qvpvlpig
SPdxsp	563	meegc-lmggfgsav----aealmdnnvl----vplkrlg
TMdxsp	536	veeamki-ggfgsfv----aqrlqemgwqg----kivnl
ECdxsp	556	veena-imggagsgvnevlmahrkpvplni-g-----
NMdxsp	567	leena-eggagsav----levlakhgickp-vlll---g
HIdxsp	556	leena-iqggagsav----aevlnssgksta-llql---g
PFdxsp	1134	yednt-i-ggfsthf----nnyliennyitkhnlyvhniy
SSdxsp	556	vednsra-agvgasav----alalgda---dv-dvpvrrfg
HPdxsp	552	sdnyk-l-ggvasai----leflseqnilk---pvksfe

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STdxsdna	1967	lpdifqddkpekqydeaglnaanivdtvl-k-al-ryne
AAdxsp	590	vpdrfiehghkdilrnlvgidaegiekavr-d-al-kggr
BSdxsp	591	ipdrfiehghsvtalleeigltkqqvanrir-l-lm---p
CRdxsp	687	lpdryidhgdyrdqlamagltsqhiastal-t-tlgrakd
CJdxsp	582	yedkfiehghkts-----eveknlekdvnslltk-vl-kfyh
PAdxsp	592	lpdyyyvehakpsemleacglldaagiekavr-q-rl-drq-
LEdxsp	676	lpdryidhgspvdqlaeagltspshiaatvf-n-il-gqtr
MLdxsp	588	lpqefyhasrsevladlgltdqdvarrit-gwvv-afgh
MTdxsp	588	lpqefyhasrsevladlgltdqdvarrit-g-wv-----
RCdxsp	597	lpdcyidhgspeemyawagltandirdtal-a-aa-rpsk
RSdxslp	596	lpdtfidhnsaevmyataglnaadierkal-e-tl---gv
RSdxs2p	599	lpdrfieqaspedmyadaglaediaatar-g-al-argr
SPCCdxsp	593	vpdllvehaspdeskqelgltpmqmadril-e----kfgs
SPdxsp	594	vpdilvdhatpeqstvdgltpaqmaqnim-a-sl-fkte
TMdxsp	567	vedlfvphggrkellsmglldsegltktv-----l-tyik
ECdxsp	587	lpdffipqgtqeemraelglldaagmeaki-----k
NMdxsp	598	vadtvtghgdpkkllddlglslaeaverrvr-a-wl---sd
HIdxsp	587	lpdyfipqatqgealadlgltdkgieekil-n-fi-a-kq
PFdxsp	1168	lsnepiehasfkddqgevvkmdkcslnrik-n-yl-knnp
SSdxsp	587	ipeqflaharrgevladigltpveiagrigr-a-sl-pvre
HPdxsp	582	iidefimhgntalvekslgldtesltdail-k-dl-gqer
STdxsdna	2078	a----e--l--ad-----gvra*-----
AAdxsp	627	l----i-----
BSdxsp	625	p----k--t--hk-----gigs-----
CRdxsp	725	a----a--kfsls-----alqa-----
CJdxsp	616	-----
PAdxsp	628	-----
LEdxsp	713	e----a--l--ev-----mt-----
MLdxsp	626	c----g--s--gddagqygprssqtm-----
MTdxsp	621	a----a--l--gt-----gvcasdaipehld
RCdxsp	634	sv---r--i--vh-----sa-----
RSdxslp	631	e----v--l--ar-----ra-----
RSdxs2p	636	vmplrq--t--ak-----prav-----
SPCCdxsp	628	r---q--r--ig-----aasa-----
SPdxsp	631	t---esvv--ap-----gvs-----
TMdxsp	601	a----r--s--re-----gkv-----
ECdxsp	617	a----w--l--a-----
NMdxsp	633	r---d--a--an-----
HIdxsp	623	g---n--l-----
PFdxsp	1205	t-----
SSdxsp	624	-----e--p--ae-----eqpa-----
HPdxsp	619	-----

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Figure 7

```

1  cgacggccccg gtagccccgg cgcggtgca gcaccgtcag acgtccgccc
51  agaaagccgt cggaagtcaa ttcgccggg gcgaacatca gggggtcgtc
101 gggatgccgt tgcggacat caccggcag gcgcgatccc agtcttcttc
151 cgggacaaac agacgcgcg gcaatatgcc gatggagcct tcgaggacgc
201 tcatgtggac gtccaccgga aaggcgtcta tatcctcgcc ctgaaggagc
251 gcgggtggcga aggcgatgat cgtcgggtcg gtcgtgcgca acagtccctt
301 catgtcgggg acattgtcgg caacgcctcg gtttgtcgag gccggttcgt
351 cgaccgggtg gcaggatcgg gatgggattg gacgaggttt cgcaaaagcc
401 gcatgaacgg ctgcgcgcgt ggctggccga ggacatggcc gccgtcaacg
451 ggctgatccg cgagcggatg gcctcgaaac acgcgccccg cattcccgag
501 gtcacggcgc atctggtcga ggccggcggc aagcggctgc ggccgtcctt
551 gacgctcgcc gcggcgcggc tgtgcggcta cgaggggccc tatcacatcc
601 atctggccgc gacggtggag ttcatccaca cggcgacgct gcttcacgac
651 gatgtgggtg acgaaagcca ccgcgcgcgc ggcaaaccga cggcgaacct
701 gctgtgggac aacaaatcct cgggtgctgt gggcgactat ctcttcgccc
751 gcagcttcca gctgatgtgc gagaccggtc cgcttcgctg gatggacatc
801 ctgcgcaatg cctcggccac catctccgag ggcgaggtgc tgcagctgac
851 cgcggccccg gatctgcgca cgaccgagga catccacctg caggtgggtgc
901 gcggcaagac ggccgcgctc ttgcccgcgg caaccgaggt gggcggcgtg
951 gtcgcggggc tgcccgaggc gcaggtcgag gcgctccacg cctacgggga
1001 cgcgctgggg atcgccttcc agatcgtcga cgacctctc gattatggcg
1051 gcgtggatgc ccagatcggc aagaacaccg gcgacgactt ccggaacgc
1101 aagctgacgc tgccggtcat caaggcgggt gcccgagccg atgccgagga
1151 gcgcgccttc tggcagcggg tgatcgagaa gggcgaccag cgcgaggggtg
1201 acctcgagca agcccatgag atcatgtccc gccacggcgc catggaggcc
1251 gcccggcagg atgcgctccg ctgggtcacg gtggcgcgcg aggcactcgg
1301 ccagctgccg gagcaccgcg tgcgcgagat gctgcacgat ctggccgatt
1351 tcgtggtcga acgcatcgcc tgatcccttc cgggcgctct gccccggcgc
1401 agcgcaggat ccgcgctgc gccctttcg gccttcgac agtccctctg
1451 ccgcgggagg ccggcctcgc ctgagaagcc gactggccg ccggtcttcc
1501 cccgaaccgc tcccgggcct gctcggaagg cgtccgcgc aaaagcccc
1551 gcgggggggc cccaccggcg gccatcagga agagaccgtt gaagcgggcc
1601 gtcgaatcc tgtcgcgccc cccccgacc gggcggtctt ccgatccgtg
1651 ttcgctcggc gatggacagc cgttccctgt ccgttcatga tggcgccatg
1701 cagaccctta ccgttccgga ttccggcctc gccccctcct gcccgccaa
1751 aggctcggcc gcggcgtctg ccgccatctg cgcagccatg atttcgtctc
1801 ggtggtcgaa ctcggtcccc cgcccgccct cagggtcgac gtgatggcgc
1851 tggggcccaa gggcgagatc tgggtgggtg aatgcaaate ctgcgcgcg
1901 gactatcagt ccgaccgcaa gtggcagggc tatctcgact ggtgcgaccg
1951 cttcttcttc gcggtggacg aggaccagcc cgggcgctcg (SEQ ID
NO: 37)

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Figure 8

```

1  atgggattgg acgaggtttc gcaaaagccg catgaacggc tcgccgcgtg
51  gctggccgag gacatggccg ccgtcaacgg gctgatccgc gagcggatgg
101 cctcgaaaca cgcgccccgc attcccaggg tcacggcgca tctggtcgag
151 gccggcggca agcggctgcg gccgctcctg acgctcgccg cggcgcggct
201 gtgcggctac gaggggccct atcacatcca tctggccgcg acggtggagt
251 tcatccacac ggcgacgctg cttcacgacg atgtggtgga cgaaagccac
301 cgcgcgcgcg gcaaaccac ggcgaaacct ctgtgggaca acaaatcctc
351 ggtgctggtg ggcgactatc tcttcgcccg cagcttcag ctgatggtcg
401 agaccggctc gcttcgcgtg atggacatcc tcgccaatgc ctcgccacc
451 atctccgagg gcgaggtgct gcagctgacc gcggcccagg atctgcgcac
501 gaccgaggac atccacctgc aggtggtgcg cggcaagacg gccgcgctct
551 ttgccgcggc aaccgaggtg ggcggcgtgg tcgcgggctg gcccgaggcg
601 caggtcgagg cgctccacgc ctacggggac gcgctgggga tcgccttcca
651 gatcgtcgac gacctcctcg attatggcgg cgtggatgcc cagatcggca
701 agaacaccgg cgacgacttc cgcgaaacga agctgacgct gccggtcatc
751 aaggcgggtg cccaggccga tgccgaggag cgcgccttct ggcagcgggt
801 gatcgagaag ggcgaccagc gcgaggggtg cctcgagcaa gcccatgcga
851 tcatgtcccg ccacggcgcc atggaggccg cccggcagga tgcgtccgc
901 tgggtcacgg tggcgcgcgga ggcactcggc cagctgccgg agcaccgcgt
951 gcgcgagatg ctgcacgatc tggccgattt cgtggtcgaa cgcacgcct
1001 ga (SEQ ID NO:38)

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Figure 9

```
1  mgldevsqkp herlaawlae dmaavnglir ermaskhapr ipevtahlve
51 aggkrlrp1l tlaaarlcgy egpyhihlaa tvefihtatl lhddvvdesh
101 rrrgkptanl lwdnkssvlv gdylfarsfq lmvetslrv mdilanasat
151 isegevlqlt aaqdlrtted ihlqvvrqkt aalfaaatev ggvvagvpea
201 qvealhaygd algiafqivd dlldyggvda qigkntgddf rerkltlpvi
251 kavaqadaee rafwqrviek gdqregdleq ahaimsrhga meaarqdalr
301 wvtvarealg qlpehplrem lhdladfvve ria (SEQ ID NO:39)
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Figure 10

```

1  ggatcgcgca ggcctcggc cagcgccacc atcagcagca gattgccgtt
51  cggcagccgc gcgaagccgg ggttgaaggc gccaaggaca taggtcgcgt
101 cgtccacccc ctgcgcgagc ggtgagcggg tcaggctgac attgtcgggc
151 cggaagatca gataatcgtc gctcaagcgc ttgccccctc gggtttcacg
201 cccagcaacg gggtcaggcc ccgggggttc cggcttcagc gccggccttc
251 tgggcctggc ggtggtgccc gatcacctcg tcgatgatga agcgcaggaa
301 tttctcgga aattcggggc cgagatcggc atcctgcgcc agcgcgcgca
351 gccgggcgat ctgcgcctcc tcgcggccgg gatcggcggg cggcagcccc
401 gattcggcct tgtagcgccc caccgcctgg gtcaccttga accgctcggc
451 gagcatgaag acgagcgccg catcgatatt gtcgatgctc tggcgatagc
501 gggtcagcgt cgcgtcggtc atgcgaatct cctttgccgc tgcggcacgg
551 ccatgcaagc acctcttgcc tttgcaatgc acaaaggcca gaggctcgtt
601 gcatatgagc gcaaccgtcc accgcctggg ctgcggaacc cagccttcgc
651 tcgatccgat catggcgctg gtcgcccagg acatgaacct ggtgaacgcg
701 gtgatectcg atcgcatgca gtccgagatc ccgctgatcc ccgaactcgc
751 cggccatctg atcgctggcg gcggcaagcg gatgcggccg atgctgacgc
801 tcgccagcgc ccggctgctc ggctattcgg gcacgcgcca ccacaagctg
851 gcggcggcag tggagtccat ccacaaccgc acgctgctgc atgacgacgt
901 ggtcgacagc tcggacctgc gccgcggccg ccgcaccgcc aacatcatct
951 ggggcaatcc cgccagcgtg ctggctcgcg acttccgtgt cagccgctcg
1001 ttcgagctga tggctgaggc cgaaagcctc aaggcgtgc acatcctgtc
1051 gaacgccagc gcggtgatcg ccgagggcga agtcaaccag ctgaccgcgg
1101 tgcgccggat cgacctgtcc gaggatcgct atctcgacat catcggcgcc
1151 aagactgcgg cgtgttcgc cgccgcctgc cgggtggcgg gcgtggtcgc
1201 cgagcgtccc gaggcggagg aactcgcgct cgacgcctat ggccgcaacc
1251 tcggcatcgc tttccagctg gtcgacgacg cgatcgacta tgtctcggac
1301 gcgtcgacga tgggcaagga tgccggcgac gatttccgcg aaggcaagat
1351 gacgctgccg gtggtcctgg cgtacgcgcg cggcgacgag gcggaacgcg
1401 gcttctggaa ggaagcgatt tcgggccgcc gcattctcga cgaggatttc
1451 gccgaggcga tccggctggt gcagagctgc cgcgcggtgg acgacacgct
1501 cgcccgtgcc cgccattacg gccagctcgc gatcgatgcg ctgggcggct
1551 tccgcgcctg cgaggcgaag gacgcgatgg tcgaggcggc cgaattcgcg
1601 gtggcgcgcg cctactgacg cgcgccgacc ggagcatttc cgggtggatc
1651 gcttgcgatc caaggctcgg gaaatgcgac catcaaaaag cttccgggga
1701 ttacgcctcg gtcgactttt cttcgccctc gtctcgtcg acttcgagcg
1751 cgtcttctc gtccatgtcg agcactacct cgatgccctc gacgatcagg
1801 tcgagctgct cgtagctcgc cgtcatctcg atc (SEQ ID NO:40)

```

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Figure 11

```
1  atgagcgcaa cgtccaccg cctgggctcg cgaaccacgc cttecgctcga
51  tccgatcatg gcgtggtcg cccaggacat gaacctggtg aacgcggtga
101 tcttcgatcg catgcagtc gagatcccg tgatccccga actcgccggc
151 catctgatcg ctggcgggcg caagcggatg cggccgatgc tgacgctcgc
201 cagcgcccg ctgctcggt attcgggcac gcgccaccac aagctggcgg
251 cggcagtgga gttcatccac accgcgacgc tgctgcatga cgacgtggtc
301 gacagctcgg acctgcgccc cggccgccc accgccaaca tcatctgggg
351 caatcccgcc agcgtgctgg tcggcgactt cctgttcagc cgctcgttcg
401 agctgatggt cgaggccgaa agcctcaagg cgctgcacat cctgtcgaac
451 gccagcgcg tgatcgccga gggcgaagtc aaccagctga ccgcggtgcg
501 ccggatcgac ctgtccgagg atcgctatct cgacatcatc ggcgccaaga
551 ctgcggcgct gttcgccgcc gcctgcggg tggcgggcgt ggtcgccgag
601 cgtcccgagg cggaggaact cgcgctcgac gcctatggcc gcaacctcgg
651 catcgctttc cagctggtcg acgacgcgat cgactatgtc tcggacgcgt
701 cgacgatggg caaggatgcc ggcgacgatt tccgcgaagg caagatgacg
751 ctgccggtgg tcctggcgta cgcgcgcggc gacgaggcgg aacgcggctt
801 ctggaaggaa gcgatttcgg gccgcgcgat ctcggaacgag gatttcgccg
851 aggcgatccg gctggtgcag agctgcgcgc cgggtggacga cacgctcgcc
901 cgtgcccgcc attacggcca gctcgcgatc gatgcgctgg gcggcttccg
951 cgcctgcgag gcgaaggacg cgatggtcga ggcggtcgaa ttcgcggtgg
1001 cgcgcgccta ctga (SEQ ID NO:41)
```

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Figure 12

```
1  msatvhrllgs rtqpsldpim alvaqdmnlv navildrmqs eiplipelag
51 hliagggkrm rpmltllasar llgysgtrhh klaaaavefih tatllhddvv
101 dssdlrrgrr taniiwgnpa svlvgdflfs rsfelmvae slkalhilsn
151 asaviaegev nqltavrrid lsedryldii gaktaalfaa acrvagvvae
201 rpeaeelald aygrnlgiaf qlvddaidyv sdastmgkda gddfregkmt
251 lpvvlayarg deaergfwke aisgrrisde dfaeairlvq scravddtla
301 rarhygqlai dalggfrace akdamveave favaray (SEQ ID NO:42)
```

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RSddsdna	372	atg-----ggattggac
STddsdna	605	atg-----agcgcaacc
SPddsdna	1	atgattcagtatgtatatatttaaacatatgaggaaattat
GSddsdna	1	-----
RCddsdna	1	atg-----gccatcga-
RSddsdna	384	ga-----ggtttcgcaaaagccgcat-----gaac
STddsdna	617	gtccaccgcctgggctcgcgaaaccagccttcgctcgatc
SPddsdna	41	gg-----agtcttgaaaagtcggtt-----cgac
GSddsdna	1	-----
RCddsdna	12	-----tttc---aa---gcaa-----gata
RSddsdna	409	ggctcgccgcgtggctggccgaggacatggccgcccgtca-
STddsdna	657	cgatcatggcgctggctcgcccaggacatgaacctgggtga-
SPddsdna	66	tggtcttcggttttct--actacgaaccgcaatgcttcac
GSddsdna	1	-----atgctggcctgca-
RCddsdna	26	ttctcg-ctcctg--ttgctcaagattttgcagcgatgg-
RSddsdna	448	acgggctgatccgcgagcggtatggcctcgaaaca---cgc
STddsdna	696	acgcggtgatcctcgatcgcatgcagtcgagat---c--
SPddsdna	104	atttaattaaaaacgag-----ttggaacaaatctc
GSddsdna	14	accgggcgatcatcgcccggatg----gaaagt---ccg
RCddsdna	62	accagttttattaatgaaggaatcagctccaaggt---cgc
RSddsdna	485	g--ccccgcattc-----ccgaggtca---cggcgc
STddsdna	731	---ccgctgatcc-----ccgaactcg---ccggcc
SPddsdna	135	a--ccagggattcgtaaatactgaattcaaattcagaat
GSddsdna	46	gttcccctgatcc-----cgagcttg---gcgccc
RCddsdna	99	a--ctggtcatgt-----c---agtca---gcaagc
RSddsdna	511	atctggctcgag-----gccggcgg
STddsdna	756	atctgatcgct-----ggcggcgg
SPddsdna	173	ttcttgaagagtgttctaaatattataaccattgctcaagg
GSddsdna	74	atcttgctcgcg-----gcgggagg
RCddsdna	122	atgtcgttgaa-----gcaggtgg
RSddsdna	530	caagcggctgcggccgc-----tcctgacgctcgcc
STddsdna	775	caagcggatgcggccga-----tgctgacgctcgcc
SPddsdna	213	aaaacaaatgcgtccttctcttgttttgctgatgtccaaa
GSddsdna	93	caagcgccttcgcccgc-----tgctgacgctggcc
RCddsdna	141	aaagcgcgatgcgtccga-----ttatg-tgcttgct

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RSddsdna	561	gcggcgcggtgtgc---ggctacgag---gggcc-----
STddsdna	806	agcgcccggtgctc---ggctattcg---ggcacg-----
SPddsdna	253	gctacaagcttgtgccatggtattgat---cggtccgtagt
GSddsdna	124	tccgcacgtctgtgc---ggttatcagccgggtcc-----
RCddsdna	171	g----gccgct-tat---gcctgtggt---gaaacc-----
RSddsdna	591	-----t---atcacatc
STddsdna	836	-----c---gccaccac
SPddsdna	291	gggcgacaaatatattgatgatgatgat---ttaagatc
GSddsdna	156	-----ggaccatcagcgt
RCddsdna	196	-----a---atttaaag
RSddsdna	600	cat-----ctggccgcgacggtg-----
STddsdna	845	aag-----ctggcgccgacggtg-----
SPddsdna	327	att-----ttcgacgggtcaaattcttctctctcaa
GSddsdna	169	catgtcggg---ctcgccgcctgcgtt-----
RCddsdna	205	catgcacagaagctggcgccattatt-----
RSddsdna	618	-----gagttcatccacacggcga
STddsdna	863	-----gagttcatccacaccgcga
SPddsdna	358	ttgagatttagcacaaataaccgagatgatccatatagcaa
GSddsdna	193	-----gagttcattcataccgccca
RCddsdna	232	-----gaaatgctgcatacggcga
RSddsdna	637	cgctgcttcacgacgatgtggtggacgaaagccaccgccg
STddsdna	882	cgctgctgcatgacgacgtggtcgacagctcggacctgcg
SPddsdna	398	gtttgctgcatgacgatgtgattgatcacgctaattgtccg
GSddsdna	212	cactgctgcatgatgatgtcgtggatgagagcacgttgcg
RCddsdna	251	ctctggtacatgatgatgatgtagatgagtcctggcttacg
RSddsdna	677	ccgcggcaaacccacg-gcgaacctgctgtgggacaacaa
STddsdna	922	ccgcggccgcccacc-gccaacatcatctggggcaatcc
SPddsdna	438	tagaggctcaccttcaagcaatgttgctttcgg-----ta
GSddsdna	252	tcgggggctggcttcg-gccaatgccgtgttcggcaacaa
RCddsdna	291	ccgtggcagaccaaca-gcaaatgcgacatggaataacca
RSddsdna	716	atcctcggtg----ctggtgggcgactatctcttcgcccg
STddsdna	961	cgccagcgtg----ctggtcggcgacttctgttcagccg
SPddsdna	473	atcgacggtcaatccttgcgggtaatttcattccttgacg
GSddsdna	291	ggcgtccgtg----ctggtaggtgacttctgttcgcccg
RCddsdna	330	gactgcggta----ctggtgggggattttctgattgcccg

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RSddsdna	752	cagcttccagctgatgggtcgagaccggctcg-----cttc
STddsdna	997	ctcgttcgagctgatgggtcgaggccgaaagc-----ctca
SPddsdna	513	g-gcttcga----ctgctatggccgccttcgaaatcccc
GSddsdna	327	ctcgttccagcttatgacagcagacggctcc-----ctga
RCddsdna	366	ggcatttgatctgctggttgatctggacaat-----atga
RSddsdna	787	gcgtgatggacatcctcgccaatgcctcgccaccatctc
STddsdna	1032	aggcgctgcacatcctgtcgaacgccagcgcggtgatcgc
SPddsdna	548	aagttacggagttgttagctacagtgatagcagacttggt
GSddsdna	362	aggatcatggcgatcctgtcggtatgcacggcgacaattgc
RCddsdna	401	tcctgttaaaggacttctctacaggaacctgtgagattgc
RSddsdna	827	cgagggcgaggtgctgcagctgaccgcgg--cccaggatc
STddsdna	1072	cgagggcgaagtcaaccagctgaccgcggtgcgcggatc
SPddsdna	588	tcgaggtgagtttttgcagctaaaaaata--ctatggat-
GSddsdna	402	tgaaggtgaagtccttcagatgggtcgtgc--agaacgacc
RCddsdna	441	tgaggggtgaagtattgcagttgc---agg--cacagcatc
RSddsdna	865	tgcgc---acgaccgaggacatccacc-----
STddsdna	1112	--gac---ctgtccgaggatcgcctatc-----
SPddsdna	625	--cct---tcatctttggaaataaaacaatcaaattttga
GSddsdna	440	ttacg---acgcctgtagaacgcctatc-----
RCddsdna	476	agccagatacaacagaagatattttatt-----
RSddsdna	889	-----tgcaggtggtgcgcggcaagacggccgcgct
STddsdna	1134	-----tcgacatcatcggcgccaagactgcgcgct
SPddsdna	660	ctattatattgaaaaaagttttttg-aaaacagccagttt
GSddsdna	464	-----ttgaagtcattcacggcaagacggctgcgct
RCddsdna	503	-----tacagattattcacggtaaaacctcacggtt
RSddsdna	920	ctttgccgcggcaaccgaggtgggcggtggtgcg-----
STddsdna	1165	gttcgccgcgcgctgccgggtggcggtggtgcg-----
SPddsdna	699	aattttcca-----aaagctgcaaggcttctacaatcct
GSddsdna	495	gtttgcggtgcctgccgtgtcggcgctgtcgtgg-----
RCddsdna	534	gttcgaactggcgaccgaaggcgctgcaatactgg-----
RSddsdna	955	cgggc---gtgcccaggcgaggtcgagggcgtccacgc
STddsdna	1200	ccgag---cgtcccaggcgaggaactcgcgctcgacgc
SPddsdna	732	cggacaatgttctcctactgtagcaacagctgctgga-ga
GSddsdna	530	ccgag---cgtccggaagcagaagaggaagctctggagcg
RCddsdna	569	caggc---aacctga-----ataccgtgaacctttacgt

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RSddsdna	992	c---tacggggacgcgctggggatcgccctccagatcgtc
STddsdna	1237	c---tatggccgaacctcggcatcgctttccagctggtc
SPddsdna	771	a---tacggtcgatgcattgggtactgcttttcaactaatg
GSddsdna	567	g---tttggcaccaatctgggtatggcggtccagcttggt
RCddsdna	601	cgttttgcccggacactttggcaat-gcttttcagattatt
RSddsdna	1029	gacgacctcctcgattatggcggcggtg-gatgcccgatc
STddsdna	1274	gacgacgcgatcgactatgtctcggac-gcgtcgcagcatg
SPddsdna	808	gatgacgtgttggtactat-acgtcgaaagatgatacttta
GSddsdna	604	gatgatgccctggattatgccgcagac-cagcaggttttg
RCddsdna	640	gatgatattctggattacacttcagat-gctgatacgcctc
RSddsdna	1068	ggcaagaacaccggcgacgacttcc-gcgaacgcaagctg
STddsdna	1313	ggcaaggatgccggcgacgatttcc-gcgaaggcaagatg
SPddsdna	847	ggaaaggcggctggtgcagatttgaagctaggggttgcta
GSddsdna	643	ggcaagaccgttggtgatgacatgc-gtgaaggcaagatc
RCddsdna	679	ggcaaaaatattggcgatgacttga-tggaaggcaaaccc
RSddsdna	1107	acgctgccggtcatcaaggcgggtggcccaggccgatgcc-
STddsdna	1352	acgctgccggtggtcctggcgtagcgcgcggcgacgag-
SPddsdna	887	cagct-cccgtcctctttgc-atggaaaaagt--atcca-
GSddsdna	682	accctgccggtcct-----ggccgcctatgaggctggct
RCddsdna	718	accctgccgctgattgcagcaatgcaaaatactcaaggt-
RSddsdna	1146	-----gaggagcgcgccttctggcagcgggtgatcgagaa
STddsdna	1391	-----gcggaacgcggcttctggaaggaagcgatttcg--
SPddsdna	922	-----ga-----acttgggtgca---atgattgtgaa
GSddsdna	716	cgccggaagatcgatattttctgggagcgcgtcattggaga
RCddsdna	757	-----gaacagcgcgcacctgatccgtcgc-----agca
RSddsdna	1181	gggcgaccagcgcgagggtgac--ctcgagcaagcccatg
STddsdna	1424	----ggccgcgcgatctcggac--gaggatttcgccgagg
SPddsdna	945	tagattcaatcatccttctgat--atccaacgggctcggt
GSddsdna	756	aggggagcagactgaggacgat--ctgcctcatgctctga
RCddsdna	785	ttgccactggcg-gtacttcacagcttgaacaagttattg
RSddsdna	1219	cgatca-----tgtcccgcacggcgccatggaggc--c
STddsdna	1458	cgatccggctggtgcagagctgccgcgcgggtggacga--c
SPddsdna	983	cttttg-----ttgagtgcactgatgctatcgagca--a
GSddsdna	794	acctga-----ttgcaaagacgggtgcgatcaatacgac
RCddsdna	824	cgattg-----tacaaaattcgggagcgcgtgga-----

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RSddsdna	1251	gcccggcaggatgcgctccgctgggtcacggtggcgcgcg
STddsdna	1496	acgctcgcccggtgcccgccattacggccagctcgcgatcg
SPddsdna	1015	accatcacttgggcaaaagaatatcaaaaaagccaaag
GSddsdna	828	gatcgcccg--cgcgaggtctatgccgacgcagctgttg
RCddsdna	852	ttattgccataagcgtgctactgaagaaaccgagcgagca
RSddsdna	1291	----aggcactcgccagctgccggagcaccgctgcgcg
STddsdna	1536	----atgcgct-gggcggttcc-gcgctgcgagggcgaa
SPddsdna	1055	----attcccttctgtgtctccctgattcacctgcaagga
GSddsdna	866	----aagccctgtccattttcccgatagcgaactgcgcc
RCddsdna	892	ttacaggcactagaaatattacctgagagtacttaccggc
RSddsdna	1327	agatgc--tgacgatctggccgatttcgtggtcgaaacgc
STddsdna	1570	ggacgcgatggtcgaggcggtcgaaattcgcggtggcgcg
SPddsdna	1091	aggcac--tttttgcgttggctgataaagtaataacgaga
GSddsdna	902	gccttc--tgatcgaaacgggttcagttcacgggtgaatcgg
RCddsdna	932	aggcgc--tggttaacttgacccgcttagctttagaccga
RSddsdna	1365	atcgccctga
STddsdna	1610	gcctactga
SPddsdna	1129	aagaagtga
GSddsdna	940	gcccgctaa
RCddsdna	970	atccaataa

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RSddsp	372	-----mgldevsq-----kphe
STddsp	605	msatv-----hrlgsrtq-----psld
SPddsp	1	miqyvylkhmrklwslgkvrstvlrfsttn
GSddsp	1	-----
RCddsp	1	-----maidf-----kq
RSddsp	408	rlaawlae-dmaavnglirermaskhapri
STddsp	656	pimalvaq-dmnlvnavildrmqse-ipli
SPddsp	31	rnashliknelegispqirg-mlnsnsefl
GSddsp	1	-----mlacnraiiarmesp-vpli
RCddsp	8	dilapvaq-dfaamdqfinegisskva-lv
RSddsp	495	pevtahlveaggkrlrplltla---aarlc
STddsp	740	pelaghliagggkrmrpmltla---sarll
SPddsp	60	eeckyytiaaggkqmrpslvllmskatslc
GSddsp	20	pqlgahlvaaggkrlrplltla---sarlc
RCddsp	36	msvskhvveaggkrmrpimc11---aayac
RSddsp	576	-----gye-gp-
STddsp	821	-----gys-gt-
SPddsp	90	hgidsrvvgdkyidddlrstgqi-lp-
GSddsp	47	-----gyqpgpd
RCddsp	63	-----get-nl-
RSddsp	591	--yhih-laatevefihtatllhddvvdesh
STddsp	836	--rhhk-laaavefihtatllhddvvdssd
SPddsp	118	--sqlr-laqitemihiasllhddvidhan
GSddsp	54	hqrhvg-laacvefihtatllhddvvddest
RCddsp	68	--khaqklaaiiemlhtatlvhdddvdesg
RSddsp	672	rrrgkptanllwdnksssvlvgdylfarsfq
STddsp	917	lrrgrrtaniiwgnpasvlvgdflfsrsfe
SPddsp	145	vrrgspssnvafgnrrsilagnfilarast
GSddsp	83	lrrglasanaavfgnkasvlvgdflfarsfq
RCddsp	96	lrrgrptanatwnnqtavlvgdfliarafd
RSddsp	762	lmvetgslrvmdilanasatisegevlqlt
STddsp	1007	lmveaeslkalhilsnasaviaeagevnqlt
SPddsp	175	amarlrnpqvtellatviadlvrgeflqlk
GSddsp	113	lmtadgslkvmailsdsatiaeagevlqmv
RCddsp	126	llvldldnmillkdfstgtceiaeagevlqlq

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RSddsp	852	aaqdlrtte-----dihlgvvrqktaalf
STddsp	1097	avrridlse-----dryldiigaktaalf
SPddsp	205	ntmdpssleikqsnfdyyieksflktasli
GSddsp	143	vqndlttpv-----erylevihgktaalf
RCddsp	156	aqhqpdttte-----diylqiihgktsrlf
RSddsp	924	aaatevggvvagvpeaqvealhaygdalgi
STddsp	1169	aaacrvagvvaerpeaeelaldagrnlg
SPddsp	235	sksckastilgqcsptvataageygrcigt
GSddsp	167	aaacrvgavvaerpeaeaealerfgtnlgm
RCddsp	180	elategaailagkpeyr-eplrffaghfgn
RSddsp	1014	afqivddlldyggvdaqigkntgddfrerk
STddsp	1259	afqlvddaivyvsdastmgkdagddfreqk
SPddsp	265	afqlmddvldytskddtlgkaagadlklgl
GSddsp	197	afqlvddaldyaadqqvlgktvgddmregk
RCddsp	209	afqiiddildytsdadtlgknigddlmegk
RSddsp	1104	ltlpvikavaqadaeerafwqrviiekdgq-
STddsp	1349	mtlpvvlavayargdeaergfwkeaisgrri-
SPddsp	295	atapvlfa-----wkkypelgami
GSddsp	227	itlpvlaayeagspedrifwervigegeq-
RCddsp	239	ptlpliaamqntqgeqrdlirrsiatggt-
RSddsp	1191	-----regdleqahaimsrhgameaarqda
STddsp	1436	-----sdedfaeairlvqscravddtlara
SPddsp	314	vnrfnhpsdiqrarslvectdaieqtitwa
GSddsp	256	-----teddlphalnliaktgainttiara
RCddsp	268	-----sq--leqvaiavqnsqaldychkra
RSddsp	1266	lrwvtvarealgqlpehplremhlhdladv
STddsp	1511	rhygglaidalggfraceakdamveavefa
SPddsp	344	keyikkakdsllclpdsparkalfaladv
GSddsp	281	qvyadaavealsifpdselrrllietvqft
RCddsp	291	teeteralqaleilpestyrqalvnltrla
RSddsp	1356	veria*
STddsp	1601	varay*
SPddsp	374	itrkk-
GSddsp	311	vnrar-
RCddsp	321	ldriq-

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```

Hidxsp 1 mtnnmnnypllslnspedlrllnkdpqlpqlcqlrayllesvsqtsghl
Ecdxsp 1 msfdiakpytlalvdstqelrllpkesslpklcdelrrylldsvsrssghf
Hpdxsp 1 -----milqnktfdlnpndiaglelvcqtlrnrilevvsangghl

Hidxsp 51 asglgtveltvalhyvyktpfdqliwdvghqayphkiltgrreqmstirq
Ecdxsp 51 asglgtveltvalhyvyntpfdqliwdvghqayphkiltgrrdkigtirq
Hpdxsp 41 ssslgavelivgmhalfdcqknpfifdtshqayahklltgrfesfstlirq

Hidxsp 101 kdgihpfpwreesefdvlsvghsstsasaglgiaavaaerenagrktvcvi
Ecdxsp 101 kggllhpfpwrgeseydvlsvghsstsasagigiavaaakegknrrtvcvi
Hpdxsp 91 fgglsqftkpsesaydyfiaghsstsvsigvgvakafrlkqtlgmpiall

Hidxsp 151 gdgaitagmafealnhagalhtdmlvilndnemsisenvgalnnhlarif
Ecdxsp 151 gdgaitagmafeamnhagdirpdmvilndnemsisenvgalnnhlaql1
Hpdxsp 141 gdgsisagifyealne1gdrkypmimilndnemsistp1galskalsqlm

Hidxsp 201 sgslystlrdgskkildkvppiknfm-kkteehmkgvmfsestlfeelg
Ecdxsp 201 sgklysslreggkkvfsgvppikell-krteehikgmvv--pgtlfeelg
Hpdxsp 191 kgpfyqsf1rskvkkilstlpesvnylasrfeesfk--litp-gvfffeelg

Hidxsp 250 fnyigpvdghnidelvattlnmrnlkgpqflhiktkkgkgyapaekdpig
Ecdxsp 248 fnyigpvdghdvlglittlknmrdlkgpqflhimtkkgryepaekdpit
Hpdxsp 238 inyigpinghdlg1ti1etlklakelkepvlihaqtlkgkgykiaegryek

Hidxsp 300 fhgvpkfdpisgelpknnsk-ptyskifgdwlcemaekdakiigitpamr
Ecdxsp 298 fhavpkfdpssgclpkssgglpsyskifgdwlcetaakdnklmaitpamr
Hpdxsp 288 whgvgpfdldtglskksksatlspteaysntl1lelakkdekivgvtaamp

Hidxsp 349 egsgmvfe1sqrfpkqyfdvaiaeqhavl1fatglaiggykpvvaiystflq
Ecdxsp 348 egsgmvfe1srkfpdryfdvaiaeqhavl1faaglaiggykpivaiystflq
Hpdxsp 338 sgtgldklidaypl1rffdv1aiaeqhaltsssamakegfkpfvsiystflq

Hidxsp 399 raydqlihdvaiqnlpvlfaidragivgadgathqgafdisfmrcipnmi
Ecdxsp 398 raydqv1hdvaiqklpvlfa1dragivgadgqthqgafdl1sylvrcipemv
Hpdxsp 388 raydsivhdacisslpiklaidragivgedgethqqgll1dv1sylvrsipnmv

Hidxsp 449 imtpsdenecrqmlytgyqcgk-paavryprgn-avgvkltp1lemlpigk
Ecdxsp 448 imtpsdenecrqmlytgyhyndgpsavryprgn-avgveltp1leklpigk
Hpdxsp 438 ifaprdnetlknavyfanehdsspcafryprgsfalkegvfepsqgfv1gr

```

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Figure 15 (page 2 of 2)

Hidxsp 497 srlirkgqkiaailnfgtllpsa--lelsek---lnatvvdmrfvkipdie
Ecdxsp 497 givkrrgeklailnfgtlmpea--akvaes---lnatlvdmrfovkldea
Hpdxsp 488 sellkkegeilligyngvgrahlvqlalkekiecalldlrflkpldhn

Hidxsp 542 minvlaqthdylvtleenaiqqgagsavaevlnssgkstallqlglpdyf
Ecdxsp 542 lilemaashealvtveenaimggagsgvnevlmahrkpvplniglpdff
Hpdxsp 538 l-saiiapqklyvfsdnyklggvasaileflseqnilkpvsfeitdef

Hidxsp 592 ipqatqqealadlgldtkgieekilnfiakqgnl
Ecdxsp 592 ipqgtqeemraelgldaagmeakikawla-----
Hpdxsp 587 imhgntalvekslgldtesltdailkdldgqer--

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Figure 16

```

Rpodsp  1  --mniivkiqqnlkdevtqlndliisclksdaeliekvgkylveaggkri
Ecopp   1  mnlekinel---taqdmagvnaaileqlnsdvqlinqlgyyivsgggkri
Gsddsp  1  -----mlacnraiiarmesvpplipqlgahlvaaggkrl
Rcsdsp  1  maidfkqdilapvaqdfaamdqfinegisskvalvmsvskhvveaggkrm

Rpodsp  49  rplltiitakmfdykgn----nhiklasavefihaatlhhddvvdnstlr
Ecopp   48  rpmiavlaaravggyegna----hvtiaaliefihtatlhhddvvdndmr
Gsddsp  35  rplltlasarlcgyqpgpdhqrhvglacvefihtatlhhddvvdnstlr
Rcsdsp  51  rpimcllaayacg-etnlkhaqk--laaiiemlhtatlhhddvvdndglr

Rpodsp  95  rfkptanviwgsksilvgdflfsqsfkmlvasgcikamvnlakasviis
Ecopp   94  rgkatanaafgnaasvlvgdfiytrafqmmtslgslkvlevmseavnvia
Gsddsp  85  rglasanavfgnkasvlvgdflfarsfqlmtadgslkvmailsdasatia
Rcsdsp  98  rgrptanatwnnqtavlvgdfliarafdllvdldnmillkdfstgtceia

Rpodsp  145 egevvqlvklnerriitideyqqivksktaelfgaacevgaiiaeqvdrv
Ecopp   144 egevlqlmnvndpdi-teenymrviysktarlfeaaaqcsgilagctpee
Gsddsp  135 egevlqmvvqndltt-pverylevihgktaalfaaacrvgavvaerpeae
Rcsdsp  148 egevlqlqaqhqpdt-tediylqiihgktsrlfelategaailagkpe-y

Rpodsp  195 skdvqnfggrllgtifqviddlldylgsdkqvgknigddflgkvltplif
Ecopp   193 ekglqdygrylgtafqliddlldynadgeqlgknvgddlnegkptlpllh
Gsddsp  184 eealerfgtnlgmafqlvddaldyaadqqvlgktvgddmregkitlpvla
Rcsdsp  196 replrrfaghfgnafqiiddildytsdadtlgknigddlmegkptlplia

Rpodsp  245 lyhkleqdkqlwlenmlksdk--rtkddfvkirdlmlkhaiynetvnys
Ecopp   243 amhhgtpegaqmirtaieqgngrhllepveamnac---gslewtrqrae
Gsddsp  234 ayeagspedrifwervi--gegeqteddlphalnliaktgainttiaraq
Rcsdsp  246 amqntqgeqrldlirrsiatggtssqle----qviaivqnsqaldychkrat

Rpodsp  293 sleneannllnkipvqniykyyfysiirfilyrsy
Ecopp   290 eeadkaiaalqvlpdtpw-realiglahaivqrdr
Gsddsp  282 vyadaavealsifpdsel-rrllietvqftvnrar
Rcsdsp  292 eeteralqaleilpesty-rqalvnltrlaldriq

```

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Figure 17

```

Rpodsp 1 -----mniivkiqqnlkdevtqlndliisclksdaeliekvgkylve
Ecodsp 1 -----mnlekineltaq---dmagvnaaileqlnsdvqlinqlgyyivs
Hiodsp 1 mkkqdlmsideiqkladp---dmqkvngnilaqlnsdvpligqlgyiyivq
Gsddsp 1 -----mlacnrailiarmespvplipqlgahlva
Rcsdsp 1 -----maidfkqdilapvaqdfaamdqfinegisskvalvmsvskhvve

Rpodsp 43 aggkrirp1ltiitakmfdykgn----nhik-lasavefihaatl1lhddv
Ecopp 42 gggkrirpmiavlaaravgyegna----hvt-iaaliefihtatl1lhddv
Hiods 142 gggkrirpliavlaarslgfegsn----sit-catfvefihtatl1lhddv
Gsddsp 29 aggkrlrplltlasarlcgyqpgpdhqrhvg-laacvefihtatl1lhddv
Rcsdsp 45 aggkrmrpimc1laayac----getnlkhaqklaaiiemlhtatl1vhddv

Rpodsp 88 vdnstlrrfkptanviwsgsktsilvgdflfsqsfklmvasgcikamnva
Ecopp 87 vdesdmrrgkatanaafgnaasvlvgdfiytrafqmmtslgslkvlevms
Hiods 277 vdesdmrrgratanaefgnaasvlvgdfiytrafqqlvaqleslkilsima
Gsddsp 78 vdestlrrglasanavfgnkasvlvgdflfarsfq1mtadgslkvmails
Rcsdsp 91 vdesglrrgrptanatwnnqtavlvvgdfliarafd1lvldnml1kdfs

Rpodsp 138 kasviisegevvqlvklnerriitideyqqivksktaelfgaacevgaii
Ecopp 137 eavnviaegevlqlmnvndpdi-teenymrviysktarlfeaaaqcsgil
Hiods 427 datnvlaegevqq1lmvndpet-seanymrviysktarlfevagqaaaiv
Gsddsp 128 dasatiaegevlqm1vqndltt-pverylevihgktaalfaaacrvgavv
Rcsdsp 141 tgtceiaegevlqlqahqdpdt-tediylqiihgktsrlfelategaail

Rpodsp 188 aeqvdrvskdvqnfgrllgtifqvidd1ldylgsdkqvgknigddflgk
Ecopp 186 agctpeeeekglqdygrylgtafqlidd1ldynadgeqlgknvgddlnegk
Hiods 574 aggteaqekalqdygrylgtafqlvddvldysantqalgknvgddlaegk
Gsddsp 177 aerpeaeaealerfgtnlgmafqlvddaldyaadqqvlgktvgddmregk
Rcsdsp 190 agkpeyre-plrrfaghfgnafqiiddildytsdadt1gknigddlmegk

Rpodsp 238 vtlpliflyhkleqdkqlwlenmlksd--krtkddfvkirdlmlkhaiyn
Ecopp 236 ptlpl1hamhhgtpeaqmirtaieqgngrhllep1leamnac---gsle
Hiods 724 ptlpl1hamrhgnaqqaalireaeiegggkreaidevlaimteh---ksld
Gsddsp 227 itlpvlaayeagspedr1fweri--gegeqteddlphalnliaktgain
Rcsdsp 239 ptlpliaamqntqgeqrdlirrsiatggtsqleqvaiavqns---gald

Rpodsp 286 etvnylssleneann1lnkipv--qniykyylfsiirfilyrsy-
Ecopp 283 wt---rqraeeeadkaiaalqvlpdt1pwrealiglahaivqrdr-
Hiods 865 ya---mnrakeeaqkavdaieilpeseykqalislaysvdrny*
Gsddsp 275 tt---iaraqvyadaavealsifpdselrr1lietvqftvnrar-
Rcsdsp 285 yc---hkrateeteralqaleilpestyrqalvnltr1aldriq-

```

FIG. 18

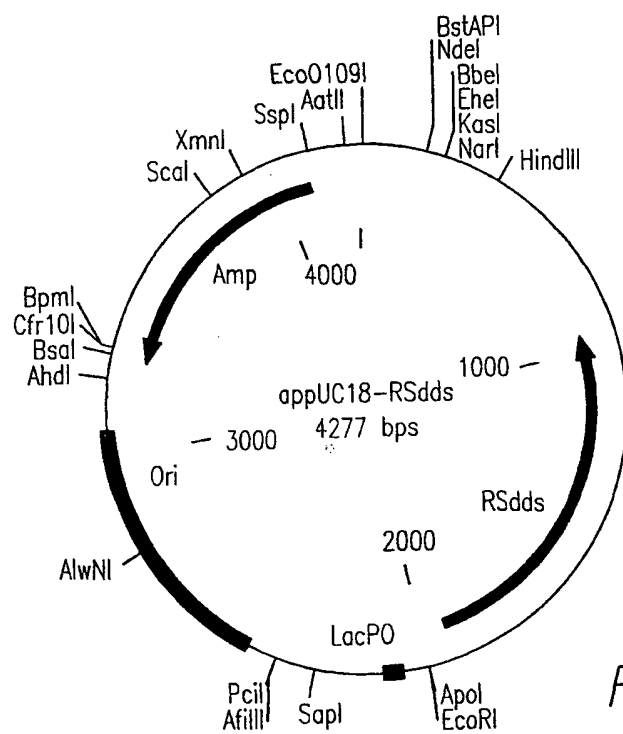
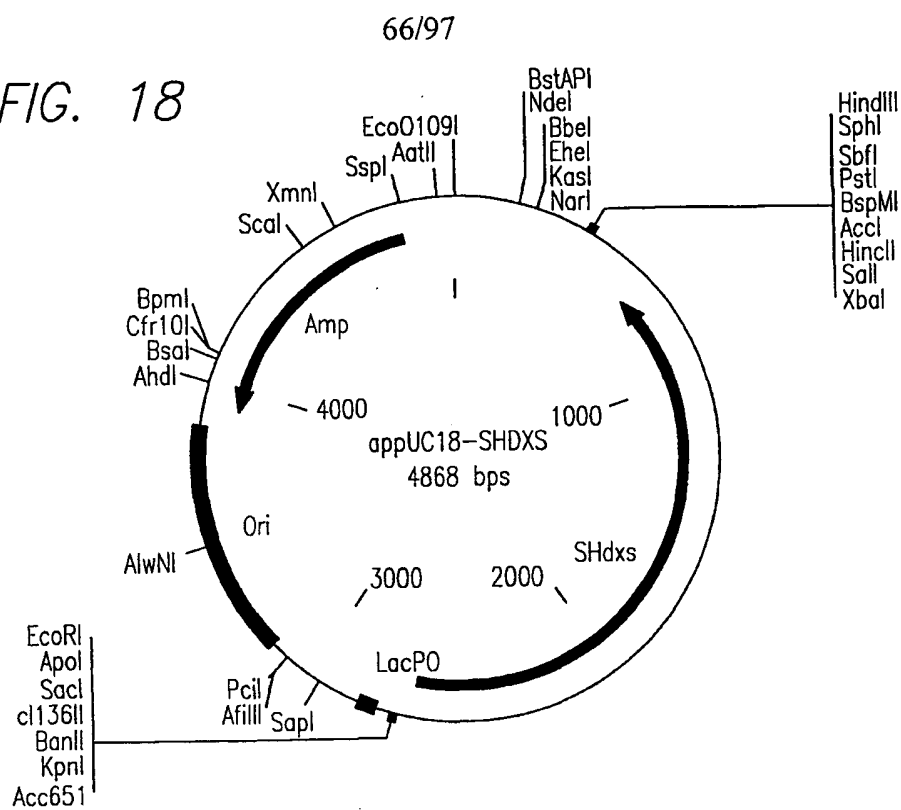


FIG. 19

FIG. 20

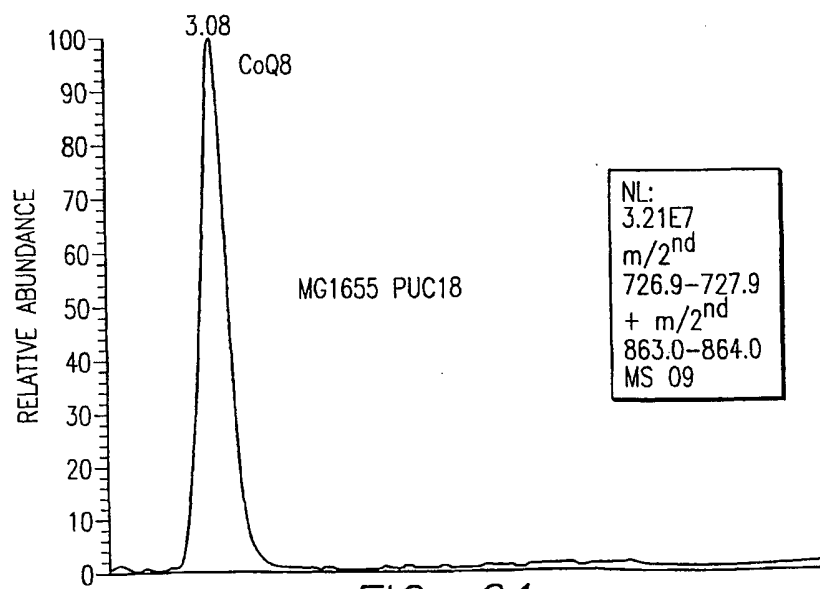
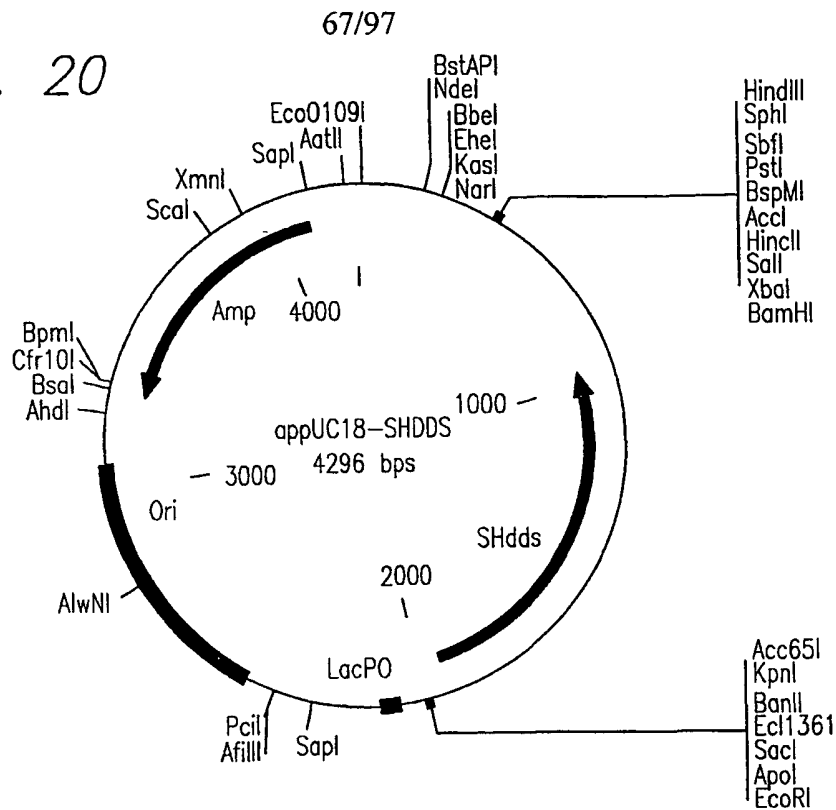


FIG. 21

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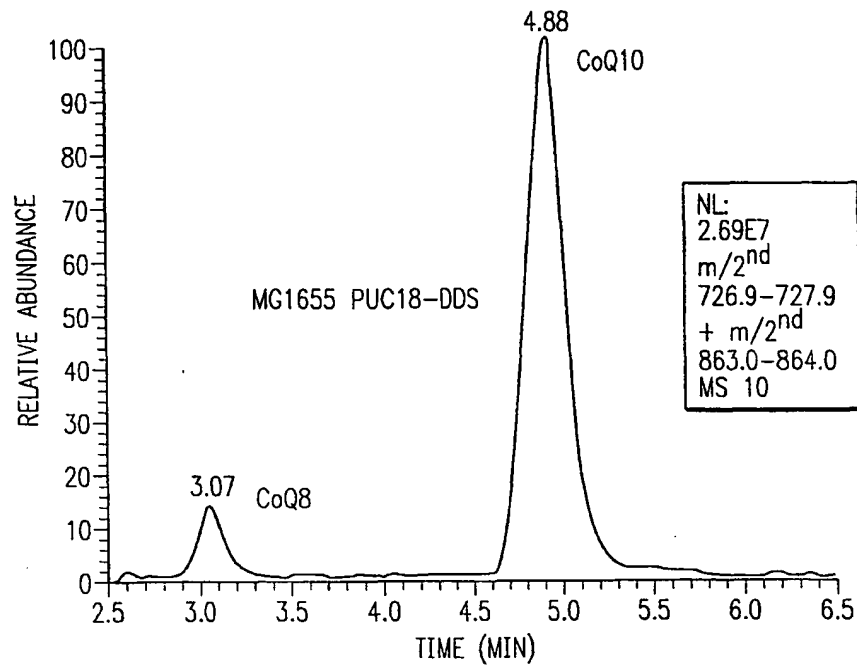


FIG. 22

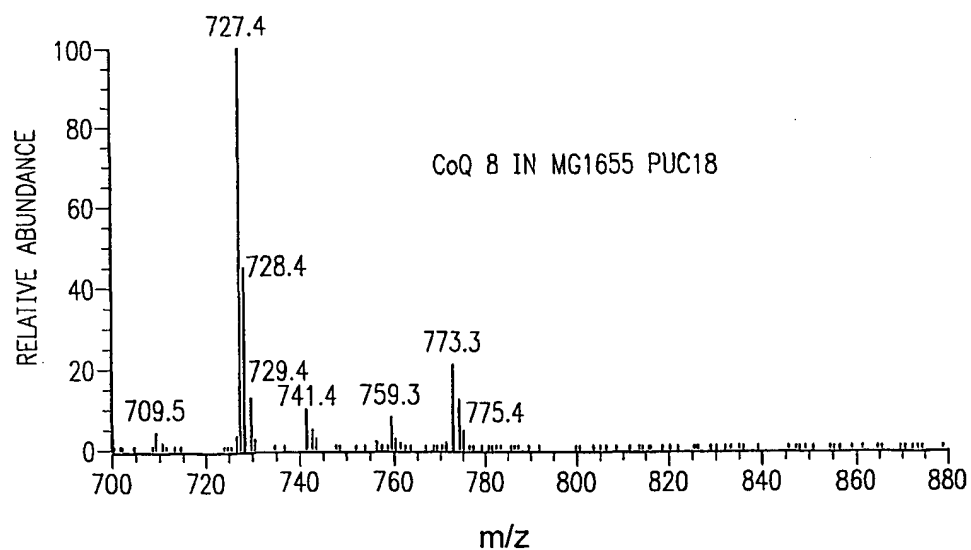


FIG. 23

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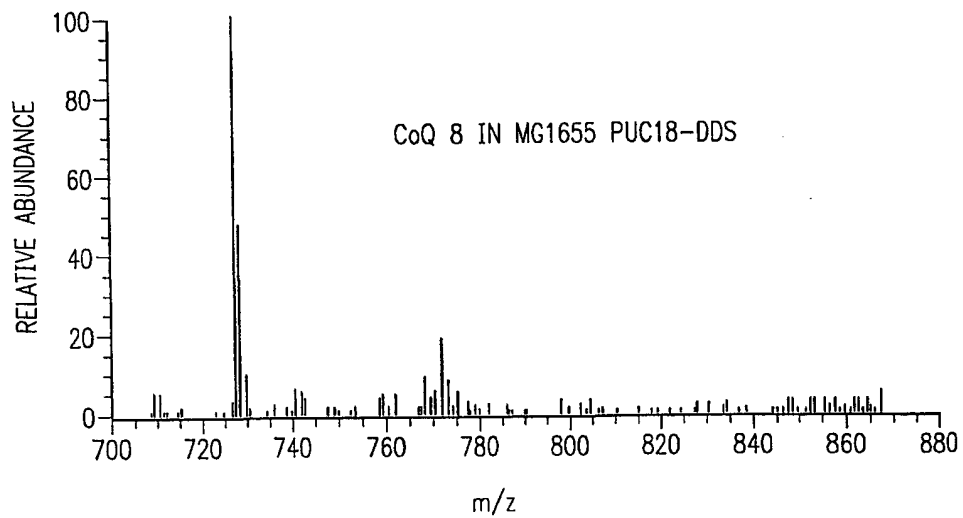


FIG. 24

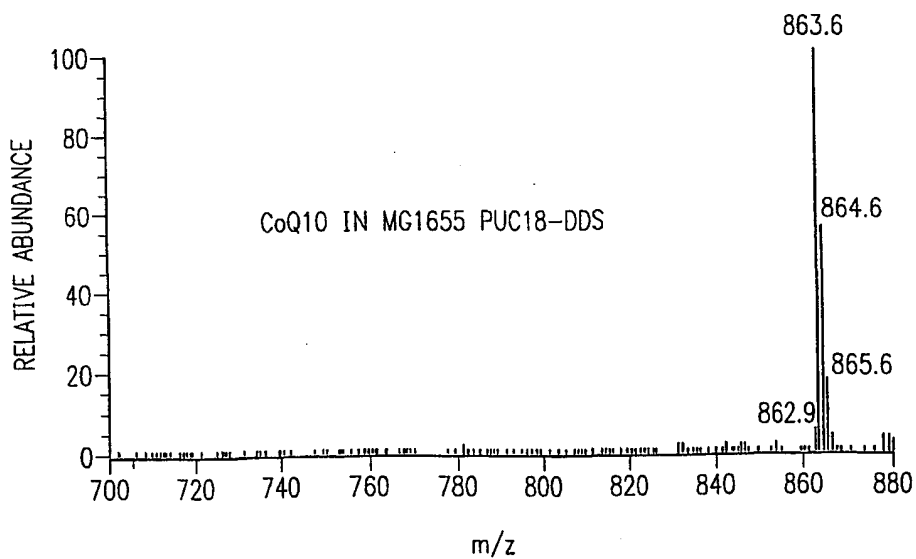


FIG. 25

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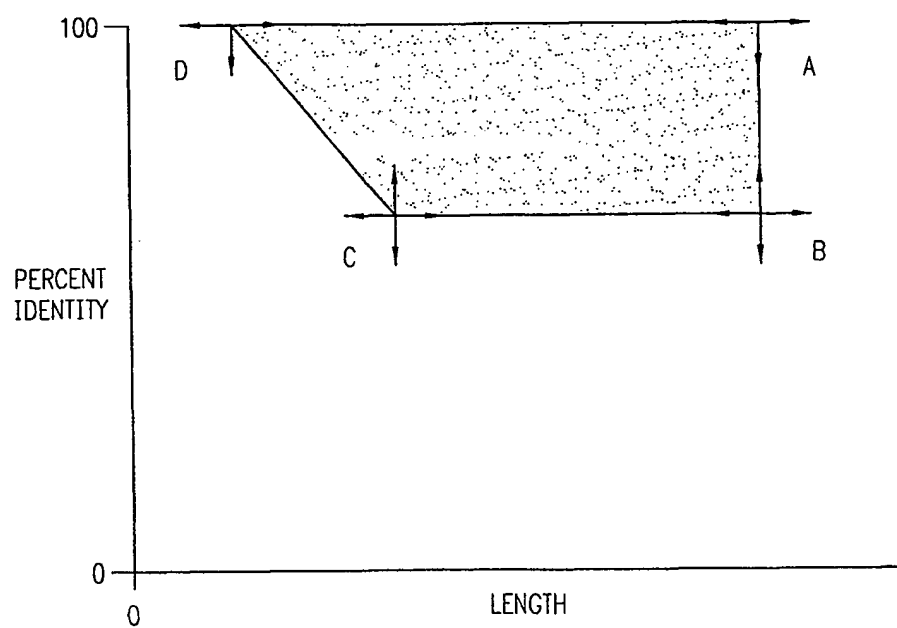


FIG. 26

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```

Bsdxrp 1 -----mknicllgatgsigeq
Hmdxrp 1 -----mqkqnivilgstgsigks
Ecdxrp 1 -----mkqltilgstgsigcs
Zmdxrp 1 -----msqprtvtvlgatgsighs
Sldxrp 1 -----mkavtllgstgsigtq
Ssdxrp 1 -----mvkrisilgstgsigtq
Mtdxrp 1 matggrvvirrrrgdnevvahndevtnstdgradgrlrvvlgstgsigtq

Bsdxrp 17 tldvllrahqdqfqlvsmisfg-rnidkavpmievfgpkfvsvglldtyhkl
Hmdxrp 19 tlvsiennpqkyhafalvgg-knveamfeqcikfrphfaalddvnaakil
Ecdxrp 17 tldvvrhnpehfrvvalvag-knvtrmveqclefspryavmddeasakll
Zmdxrp 20 tldliernldryqvialtan-rnvkdladaakrtanakraviadpslyndl
Sldxrp 17 tldileqypdrflrvglag-rnvallseqirrhprpeivaiqdaaqslsel
Ssdxrp 18 tldivthhpdafqvvglaag-gnvallagqvaefrpeivairqaekledl
Mtdxrp 51 alqviadnpdrfevvglaggaahldtllrqaqtgvtniavadehaaq--

Bsdxrp 66 kqmsfsfec---qiglgeeglieaavmeevdivvnallgsvgliptlkai
Hmdxrp 68 rekli-ahhiptevlagrraiceaahpdadqimasivgaagllptlsav
Ecdxrp 66 ktmlq-qggsrtevlsggqaacdmaaledvdqvmaaiivgaagllptlaai
Zmdxrp 69 keala---gssveaaagadalve-aammgadwtmaaiigcaglkatlaii
Sldxrp 66 qaaiadl-dnppliltgeagvtevarygdaeivvtgivgcagllptiaai
Ssdxrp 67 kaavaeltdyqpmvvgveegvvevarygdaesvvtgivgcagllptmaai
Mtdxrp 99 -----rvgdip---yhgsdaatrlveqteadvlnalvgalgrptlaal

Bsdxrp 113 eqkktialanketlvtaghivkehakkydvpllpvdsehsaifqalqg--
Hmdxrp 117 kagkrvllankeslvtcgqlfidavknygskllpvdsehnaifq---s-l
Ecdxrp 115 ragktillankeslvtcgrlfmdavkqskaqllpvdsehnaifq---s-l
Zmdxrp 115 rkgktvalankeslvsagglmidavrehgttllpvdsehnaifq---c-f
Sldxrp 115 eagkdialanketliaagpvvplllqkhgvtitpadsehsaifqciqg-l
Ssdxrp 117 aagkdialanketliagapvvplplvekmvgkllpadsehsaifqclqg-v
Mtdxrp 141 ktgarlalankeslvaggsllvraarpq--qivpvdsehsalaqclrggt

Bsdxrp 161 -eqak-----nierliitasggsfrdktreelesvtvedalkh
Hmdxrp 163 ppeaqekigfcplsel-gvskiiltgsggpfrtypleqftnitpeqavah
Ecdxrp 161 pqpqhnlgyadlegn-gvvsilltgsggpfrtprldlatmtpdqacrh
Zmdxrp 161 phhnrly-----vrriiitasggsfrttslaematvtperavqh
Sldxrp 164 sthad----frpaqvaglrillltasggafrdwpverlsqvtvadalkh
Ssdxrp 166 pe-----gglrriiiltasggafrdlpverlpfvtvqdalkh
Mtdxrp 189 pde-----vaklvltasggsfrgwsaadlehtpeqagah

```

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Figure 27 (page 2 of 2)

Bsdxrp 198 pnwsmgakitidsatmmnkglevieahwlf dipyeqidvvlhkesiihsm
 Hmdxrp 212 pnwsmgkksivsdsatmmnkgleyiearwlf nasaeemeviihpqsihsm
 Ecdxrp 210 pnwsmgrkisvdsatmmnkgleyiearwlf nasasqmevlihpqsvihsm
 Zmdxrp 200 pnwsmgakisidsatmmnkglelieayhlfqiplekfeilvhppqsvihsm
 Sldxrp 210 pnwsmgrkitvdsatlmnkglevieahylfgldydyidivihpqsiihsl
 Ssdxrp 202 pnwsmgqkitidsatlmnkglevieahylfgldydhidivihpqsiihsl
 Mtdxrp 224 ptwsmgpmntl nsaslvnkgleviethllfgipydridvvvhppqsihsm

Bsdxrp 248 vefhdksviaqlgtpdmrvpiqyaltypdrlplpdakrlelweigslhfe
 Hmdxrp 262 vryvdgsvitqmgnpdmrt piaetmayphrtfa-gvepldffkikeltfi
 Ecdxrp 260 vryqdgsvlaqlgepdmrt piahtma wpnr vns-gvkpldfcklsaltfa
 Zmdxrp 250 veyldgsilaqigspdmrt pightlawpkrmet-paesldftklrqmdfe
 Sldxrp 260 ieledtsvlaqlgwpdmrlpllyalswpdrlst-qwsaldlvkagslefr
 Ssdxrp 252 ievqdtsvlaqlgwpdmrlpllyalswperiyt-dwepldlvkagslsfr
 Mtdxrp 274 vtfidgstiaqasppdmklpislal gwprv-sgaaaacdfhtasswefe

Bsdxrp 298 kadfdrfrclqfafesgkiggtmpvlnaanevavaaflagkipflaied
 Hmdxrp 311 epdfnrypnklklaidafaagqyattamnaaneia vqafldr qigfmdiak
 Ecdxrp 309 apdydrypc lklameafegqgaattalnaaneitvaaf laqqirftdiaa
 Zmdxrp 299 apdyerfpaltlamesiksggar pavnmaaneia vaafl dkkigfldiak
 Sldxrp 309 epdhakypcmdlayaagrkggtmpavlnaaneqaval fleeqihfsdipr
 Ssdxrp 301 epdhakypcmqlaygagraggampavlnaaneqaval flqekisfldipr
 Mtdxrp 323 pldt dvfpavelarqagvaggcmtavynaane eaaaaf lagrigfpaivg

Bsdxrp 348 cieka--ltrhqllkkpswr---tfkkwtk-----ipgdtsiqysh
 Hmdxrp 361 inskt--ierispytiqniddvleidaqare-----ia-ktllre--
 Ecdxrp 359 lnlsv--lekmdmrepqcvddvlsvdanare-----varkevmlas
 Zmdxrp 349 ivekt--ldhytpatpssledvfaidnear-----iqaaalmeslp
 Sldxrp 359 lieracdrhqtewqqpsl ddilaydawarqfv-----qasyqslesvv
 Ssdxrp 351 liektcdlyvgqntaspdletilaadqwarrtv-----lensacvatrp
 Mtdxrp 373 iiadvlhaadqwavepatvddvldaqrwareraqravsgmasvaiastak

Bsdxrp 384 kvvcs-----
 Hmdxrp 398 -----
 Ecdxrp 399 -----
 Zmdxrp 388 a-----
 Sldxrp 403 -----
 Ssdxrp 395 -----
 Mtdxrp 423 pgaagrastlers

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Figure 28

ggccccgggctggtgggggtttctggcgctggggctggtgttcggcgcggttcttcttcgtcg
cgatcgtgacgcggaacgccaagctggcgcgggggcaggtctatgtcgggctgccggtgc
tcgcgctgctgctgctccgcgaccatccgcagggctttgcgcgacgctgtggacgatgg
cgatcgtctgggtgtgcgacagcggcgctatatttgcgggtcgcgcgatcgggtgggccc
agctcgcgcccccgatcagcccgaacaagacctggcgggggctgatcggcggggttggttg
ccgcgacccgttctccgcggctatgtcgcgctggcgcggggagcgcgatcggctggt
ggctggtcgcggtgtcgcgctggtagccttcgcctcgcagatcggcgacctgtacgaga
gccatctcaagcgggtcgcgggcggtgaaggattcgagcaacctgctgcccgccatggcg
gcattctcgaccggctcgacggccttgtcttcgcagccccgggtgcagctttgttttttg
cgatccatcatcaggtggtcgtgggaggatactggtggtgaagcgctcacggtgttggg
ggcgaccggctcggtcggcacctcgacgctggatctgatcgaacgaaatccgcacgcctt
cgaagtctgtggcgctgaccgcaaattgcgatgtcgagaagctggctgcccgcgcatccg
cacgcgcgcgcgctgcgcgctggcgcgacgagaaatgcctgccggcgctacaggagcg
gctggcgcgcgagcggtgtcgagggcgatggcgggggcgcatcgggtgtcgacgtggcgcg
gatgggtgctgactggacgatggctgcgatcgtcggcgagcgagggctcaagccggtgat
ggcgcgctggaggccggtggcacccgtcgcgctcgcgaaacaaggagtgcctcgtctcggc
gggtgaggtgatgatggcgggcgcccgcgcgcatggcgcgacgctgctgccggtcgattc
ggagcacaatgcgggtgttccagtgcctcgatcgacccgcgcccagggcgctccgcgggat
catccttaccgccagcggtggtccgttccgcgcgacgcccgaaggaagcgatgcgcgacat
cacccccgcacaggcggtggcgcatcccaactggtcgatggcgccaagatctcggtcga
ctccgcgacgatgatgaacaaggggctcgaactgatcgaagccttccacctgttcccggt
cgccgcgagcaactggcgtgctggtccatcgccaatccgtcgtccattcgatggtgga
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ctatgcgctggcttggcccagcggtggagacgctgtgcccgcgctcgacctgccac
ggtgggtaagctcgagttcgaaaatcccgatctcgatcgttcccggcgctcgcgctggc
gatggaggcattgaaggcggggcgggcgctccggccattctcaatgccgccaacgaagt
cgccgtcgcggcctttctcgcggggcggtatcggttcttgaattgccgcaatctctgc
cgatacgctgtctcgctatgacccggcgcgccggaaacgctcgatgccgtgctggcgat
cgacgcggaggcgcggtttacgcggctgagcgagtgaaggactgcgtcgcttgatcaa
tcccccgcatcctgctcaccattctggcgcttcgcgctggtgatcggggcgctcgtgttc
ctgcacgagctgggacattatctggcgggcgccctcttcgggggtgaaggccgaggaattc
tcgatcggcttcggccgcgagatcgccggcaccaccgatcgccgcggcacgcgctggaag
ttcagcctgttgccgctggcggtatgtccgcttcgcggcgacatgaacccggcgagc
cagccttcgcccgaatggctgcagaccagcccggcc (SEQ ID NO: 95)

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Figure 29

gtggtgaagcgcgtcacggtgttgggggacgggctcggtcggcacctcgacgctggat
ctgatcgaacgaaatccgcacgccttcgaagtcgtggcgctgaccgcaaattgcgatgtc
gagaagctggctgccgcggcgatccgcacgcgcgcgcgtgcgccgtggtcgccgacgag
aaatgcctgccggcgctacaggagcggctggccggcagcgggtgtcgaggcgatggcggg
gcgcatcgggtgtgcgacgtggcgcggtgggtgctgactggacgatggctgcgatcgtc
ggcagcgcagggctcaagccgggtgatggccgcgtggaggccgggtggcacgctcgcgctc
gcgaacaaggagtcgctcgtctcgccgggtgaggtgatgatggccggcgcccgcgcgcat
ggcgcgacgctgctgccggtcgattcggagcacaatgcgggtgtccagtgcccgatcgc
accgcgccagggcgctccgccggatcatccttaccgccagcgggtggctccgttcgcgcg
acgccgaagggaagcgatgcgcgacatcaccccgacagggcggtggcgcatcccaactgg
tcgatggcgccaagatctcggtcgactccgcgacgatgatgaacaaggggctcgaactg
atcgaagccttccacctgttcccggtcgccgcgagcaactggccgtgctggctccatcgc
caatccgtcgtccattcgatgggtggaatatgtcgacggatcgggtgctggcccgctcggc
acgcccacatgcgcacgccgatcgccatgcgctggcttggcccgagcggatggagacg
ctgtgcccgcgctcgaccttgccacgggtgggtaagctcgagttcgaaaatcccgatctc
gatcgcttcccgcgctcgcgctggcgatggaggcattgaaggcgggcgggcgcgctccg
gccattctcaatgccgccaacgaagtcgccgtcgcgcccttctcgccggcgcgatcgga
ttccttgaaattgccgcaatctctgccgatacgctgtctcgctatgacccggcgcgccg
gaaacgctcgatgccgtgctggcgatcgacgcggaggcgcggtttacgcggctgagcga
gtgaaggactgcgtcgcttga (SEQ ID NO: 96)

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Figure 30

```
1  vvkrvtvlga tgsvglstld liernphafe vvaltancdv eklaaaairt
51  rarcavvade kclpalqerl agsgveamgg ahsvcdvarm gadwtmaaiv
101 gsaglkpvma aleaggtval ankeslvsag evmmaarah gatllpvdse
151 hnavfqcldr taprgvrrii ltagggpfra tpkeamrdit paqavahpnw
201 smgakisvds atmmnkglel ieafhlfpva aeqlavlvhr qsvvhsmvey
251 vdgsvlaqlg tpdmrtpiay alawpermet lcppldlav gklefenpdl
301 drfpalalam ealkaggarp ailnaaneva vaaflagrig fleiaaisad
351 tlsrydpaap etldavlaid aearlyaaer vkdcva (SEQ ID NO:97)
```

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Figure 31 (page 1 of 15)

Stdxrds	1	-----
Padxrd	1	at-----
Zmdxrd	1	-----
Sgdxrd	1	-----
Nmdxrd	1	-----
Ecdxrd	1	-----
Sldxrd	1	-----
Mldxrd	1	-----
Pmdxrp	1	atgagtattagttat-----
Atdxrd	1	atgatgacattaaactcactatctccagctgaatccaaagctatttcttt
Cjdxrd	1	-----
Pfdxrd	1	-----
Stdxrds	1	-----gtgg-----
Padxrd	3	-----gagt-----
Zmdxrd	1	-----atga-----
Sgdxrd	1	-----ttgg-----
Nmdxrd	1	-----a-----
Ecdxrd	1	-----a-----
Sldxrd	1	-----g-----
Mldxrd	1	-----g-----
Pmdxrp	16	-----ttta-----
Atdxrd	51	cttggatacctccaggttcaatccaatccctaaactctcaggtgggttta
Cjdxrd	1	-----
Pfdxrd	1	-----a-----
Stdxrds	5	-----
Padxrd	7	-----
Zmdxrd	5	-----
Sgdxrd	5	-----
Nmdxrd	2	-----
Ecdxrd	2	-----
Sldxrd	2	-----
Mldxrd	2	-----
Pmdxrp	20	-----
Atdxrd	101	gtttgaggaggaggaatcaagggagaggttttgaaaaagggtgtaagtgt
Cjdxrd	1	-----
Pfdxrd	2	-----

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Figure 31 (page 2 of 15)

Stdxclds	5	-----
Padxrd	7	-----
Zmdxrd	5	-----
Sgdxrd	5	-----
Nmdxrd	2	-----
Ecdxrd	2	-----
Sldxrd	2	-----
Mldxrd	2	-----
Pmdxrp	20	-----
Atdxrd	151	tcagtgaagtgacagcagcaacaacacctccagcatggcctgggag
Cjdxrd	1	-----
Pfdxrd	2	-----
Stdxclds	5	-----tga---ag-----
Padxrd	7	-----cgaccgcag-----
Zmdxrd	5	-----gtc---ag-----
Sgdxrd	5	-----tca-----
Nmdxrd	2	-----tga---ca-----
Ecdxrd	2	-----tga---ag-----
Sldxrd	2	-----tga---aa-----
Mldxrd	2	-----tga---acaatccgatcgaggggcacgctggcgccgcct
Pmdxrp	20	-----tga---aa-----
Atdxrd	201	agctgtccctga---gg-----
Cjdxrd	1	-----
Pfdxrd	2	-----tga---ag-----
Stdxclds	10	-cg-----c-----gtca-cggtgttgggggcgacc-----
Padxrd	16	-cg-----g-----atca-gcgtgctcggcgcgacc-----
Zmdxrd	10	-cc-----aagaacagtca-ctgttttaggggcgacc-----
Sgdxrd	8	-----ttctcggtcgacc-----
Nmdxrd	7	-ccacaagtc-----ctga-ccatattaggcagtacc-----
Ecdxrd	7	-ca-----a-----ctca-ccattctgggctcgacc-----
Sldxrd	7	-gc-----a-----gtga-cactgctcggttcaacc-----
Mldxrd	39	ccg-----c-----gtgc-tggtgttgggaagtact-----
Pmdxrp	25	-aa-----g-----atcg-ttatttttaggttcaact-----
Atdxrd	215	-cg-----c-----ctcgtcaatcttgggatggacaaaacccatctc
Cjdxrd	1	-----atga-tactttttggaagtacg-----
Pfdxrd	7	-----aa-atatatattatatatt-----

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```

Stdxrds 34 -----ggctcggtcggcacctcgacgctggatc-----
Padxrd 40 -----ggctcgatcggcctgagcaccctggacg-----
Zmdxrd 40 -----ggatccattgggtcattcaacactggatt-----
Sgdxrd 22 -----ggctcgatcggcaccaggccatcgacg-----
Nmdxrd 37 -----ggcagcataggcgaaagcacgctggacg-----
Ecdxrd 31 -----ggctcgattggttgacgacgctggacg-----
Sldxrd 31 -----ggctcgatcgggacacaaaaccctagaca-----
Mldxrd 64 -----ggctcaattggcaccaggcgctggaag-----
Pmdxrp 49 -----ggatcgattggtaccagtactttatccg-----
Atdxrd 252 tatcgttggatctactggttctattggcactcagacattggata-----
Cjdxrd 22 -----ggc-----agtataggag-----
Pfdxrd 26 -----ttttct-tcatcacaataactattaatgatttag

Stdxrds 62 -----tgatcgaacgaaatccgcacgccttcgaagtcg-----tggc
Padxrd 68 -----tcgtccagcgtcatcccgatcgttacgaagcct-----tcgc
Zmdxrd 68 -----taatcgaacggaatttagatcggatcaggtca-----tcgc
Sgdxrd 50 -----tgggtgctcgcgaaccccgccggttcaaggtgg-----tcgc
Nmdxrd 65 -----ttgtctcccgccaccccgaaaaattccgcgtat-----tcgc
Ecdxrd 59 -----tgggtgcgcataatcccgaaacacttccgcgtag-----ttgc
Sldxrd 59 -----ttcttgagcagtatcccgatcgtttcgcctcg-----tagg
Mldxrd 92 -----ttatcgccgcaatccggaccggttcgaggttag-----tcgg
Pmdxrp 77 -----tgattacacataatcctgataagtaccaagtg-----ttgc
Atdxrd 296 -----ttgtggctgagaatcctgacaaattcagagttg-----tggc
Cjdxrd 35 -----taaatgctcttaaaacttgctgctttaaaaaaca-----ttcc
Pfdxrd 59 taataaataatacatcaaaatgtgtttccattgaaagaagaaaaataac

Stdxrds 99 gct-----gaccgca-----aattgc
Padxrd 105 cct-----gactggc-----ttcagc
Zmdxrd 105 ttt-----gaccgcc-----aaccgc
Sgdxrd 87 gct-----gtccgcg-----gccggc
Nmdxrd 102 gct-----ggcaggg-----cataag
Ecdxrd 96 gct-----ggtggca-----ggcaaa
Sldxrd 96 gct-----ggcggct-----ggtcgt
Mldxrd 129 gct-----ggccgc-----c
Pmdxrp 114 gtt-----agttggt-----ggacgt
Atdxrd 333 tct-----agctgct-----ggttcg
Cjdxrd 72 cat-----ttctgct-----ttagct
Pfdxrd 109 gcatatataaattatggtataggatataatggaccagataataaaataac

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```

Stdxrds 115 -----gatgtcgag--aagctgg-----c-----tgc
Padxrd 121 cgccctggccgaactcgag--gcgctg-----tgc
Zmdxrd 121 -----aatgtcaaa--gatctgg-----c-----cga
Sgdxrd 103 -----ggcgcggtg--gagctgc-----t-----cgc
Nmdxrd 118 -----caggtcgag--aaattgg-----c-----ggc
Ecdxrd 112 -----aatgtc-ac--tcgcatg-----g-----tag
Sldxrd 112 -----aatgtggcg--ctggt-----t-----
Mldxrd 139 -----gggggcgcg--cagctggacacgc-----tgc
Pmdxrp 130 -----aatgtagagctaattgtt-----c-----aac
Atdxrd 349 -----aatgttact--ctacttg-----c-----t--
Cjdxrd 88 -----tgtggggat--aacatcg-----c-----t--
Pfdxrd 159 -----aaagagtag--aagatgt-----aaaagaataaagttatgc

Stdxrds 135 -cgcg---gcgac--cgcac-g-cgcgcg-c-gctgc--g-c-----c
Padxrd 148 -ctca---ggcacc--gcccc-g-tctatgc-ggtggt-g-c-----c
Zmdxrd 141 -tgcg---gcgaaa--agaac-g-aatgcca-agcgg--g-c-----g
Sgdxrd 123 -cgag---caggccgtcgactg-ggcgtgc-acacc--g-t-----c
Nmdxrd 138 tcaat---gtcaaa--cgttc---caccgcg-aatat--g-c-----c
Ecdxrd 131 -aaca---gtgcct--ggaat-t-ctctcccgcctat--g-c-----c
Sldxrd 126 ----g---tcggag--caaat-t-cggcggc-accga--c-c-----a
Mldxrd 164 -tgag---gc-----agcgc-gccgc--gac-----c
Pmdxrp 152 -aatgtttgacatt--ccaac-c-gtcgttt-gctgc--g-ttagatgac
Atdxrd 367 -----gatc--aggta-a-ggagatt-taagcctg-c-----a
Cjdxrd 106 -cttt---taaatag--agcaa-atcgcaagg-tttaa--a-c-----c
Pfdxrd 193 -aaaa---aggat---ttaa-t-agatatt-ggtgc--a-a-----t

Stdxrds 166 gtggtcgc--cg-----ac-----ga-----gaaatgc---
Padxrd 180 ggagcagg--cc-----gc-----gg-----cgattgc---
Zmdxrd 172 gttatcgc--tg-----ac-----cc-----gtcgtt---
Sgdxrd 157 gcggtggc--cg-----acccggccgcga-----ggaagccg--
Nmdxrd 169 gtcgttgc--cg-----at-----gc-----cgaa--c---
Ecdxrd 163 gtaatgga--cg-----at-----gaagcgagtgcgaaactt---
Sldxrd 154 gagattgtggcg-----at-----tc-----aagatgcagc
Mldxrd 184 ggcgtcac--ca-----at-----atc-----gccatcg--
Pmdxrp 193 gatgtcgc--ag-----cc-----aaaatgt---
Atdxrd 394 ttggttgc--tgtagaaac-----ga-----gtcactg---
Cjdxrd 138 caaatgtg--tt-----tc-----ca-----taaaaga---
Pfdxrd 222 aaagaaac--ca-----at-----taatgta---gcaattt---

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Stdxrds 187 ----ctg-----c---cg---gc--gctacagg-----agcggctg--
Padxrd 201 ----ctt-----g---ca---gg--gct-cgct-----cgccgc-g--
Zmdxrd 193 ----tat-----a---at---ga--tctgaaag-----aggctttg--
Sgdxrd 188 ----ctg-----c---gc---ga--ggccctggcgccaaggcgag--
Nmdxrd 188 ----acg-----c---cg---cccggcttgaag-----ccctgttgaa
Ecdxrd 193 ----ctt-----aaaacg---at--gctacagc-----aacag-----
Sldxrd 180 tcagctg-----t---cg---ga--actgcaag-----cggcgatc--
Mldxrd 206 ----ctg-----a---cgatcgc--gc---gg-----ctcagctg--
Pmdxrp 212 ----tgg-----c-----agaga-----aactgaaa--
Atdxrd 421 ----att-----a---at---ga--gcttaaag-----aggcttta--
Cjdxrd 159 ----tt-----c-----aaaaaata-----agcattta--
Pfdxrd 248 ----ttggaagtac---tg--gt--agtatagg-----tacgaatg--

Stdxrds 211 ----gcc-----ggcagcgg-----
Padxrd 223 ----gcg-----ggtatccg-----
Zmdxrd 217 ----gcc-----ggaagctc-----
Sgdxrd 218 ----ggc-----gcccgtg-----
Nmdxrd 216 acgcgac-----ggca-cgg-----
Ecdxrd 217 ----gtagccg-----
Sldxrd 208 ----gca-----gaccttga-----
Mldxrd 229 ----gcc-----ggc-----
Pmdxrp 229 ----gcc-----caccaa-----
Atdxrd 445 ----gct-----gatttgga-----
Cjdxrd 178 ----gtt-----aaacacga-----
Pfdxrd 278 ----ctttaaatataataaaggagtgtaataaaattgaaaatgtttttaa

Stdxrds 222 tg-----tcg-ag-----gcgat-gggcggggc-----gca
Padxrd 234 ca-----ccc-gg-----gtgct-gttcggcga-----gca
Zmdxrd 228 tg-----ttg-ag-----gcagc-cgcgggtgc-----tga
Sgdxrd 229 cc-----gcg-g-----gtgct-ggcggggccc-----gga
Nmdxrd 230 cg-----actcag-----gtttt-acacggcgc-----gca
Ecdxrd 225 ca-----ccg-aa-----gtctt-aagtgggca-----aca
Sldxrd 219 ta-----atc-cg-----ccgct-catcctgac-----
Mldxrd 235 -g-----aca-tc-----cctta-ccacgggac-----cga
Pmdxrp 238 ag-----cca-aacaacagtctt-agcaggaca-----gca
Atdxrd 456 ctataaaactcg-ag-----attat-tccaggaga-----gca
Cjdxrd 189 ta-----gag-tt-----tttatagggaagaa-----ggg
Pfdxrd 324 tg-----tta-aa-----gcatt-gtatgtgaataagagtgatgaatgaa

```

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Stdxcds	246	ttcgg-----tgtgcgacgtggc---g-----cgga-----
Padxrd	258	ggcgt-----tgtgcgaagtggc---c-----ag-----
Zmdxrd	252	tgccct-----tggtcgaagccgc---c-----atga-----
Sgdxrd	252	cgcg-----cgaccgagctggcc---g-----cggc-----
Nmdxrd	255	ggcat-----tggttgacgttgccctctg-----ccga-----
Ecdxrd	249	agccg-----cttgcgatatggca---g-----cgct-----
Sldxrd	240	--cgg-----tgaggcaggtgtc---a-----cgga-----
Mldxrd	258	tgcg-----gtcac---c-----cggc-----
Pmdxrp	267	agcca-----tttgtgagttagc---gg-----caca-----
Atdxrd	486	aggag-----tgattgaggttgc---c-----cgac-----
Cjdxrd	214	ttagagcaaattttaacagaatgt---c-----aaga-----
Pfdxrd	361	ttata-----tgaacaagctaga---gaatttttaccagaatatttgt
Stdxcds	269	-----
Padxrd	279	-----
Zmdxrd	275	-----
Sgdxrd	276	-----
Nmdxrd	282	-----
Ecdxrd	273	-----
Sldxrd	261	-----
Mldxrd	272	-----
Pmdxrp	291	-----
Atdxrd	509	-----
Cjdxrd	243	-----
Pfdxrd	401	gtatacatgataaaaagtgtatatgaagaattaaaagaactggtaaaaaat
Stdxcds	269	-----tg-----gg--tgctga--
Padxrd	279	-----cg-----cg--cccgaa--
Zmdxrd	275	-----tg-----gg--tgccga--
Sgdxrd	276	-----gg-----ag--tgcc-a--
Nmdxrd	282	-----cg-----aa--gtcag--
Ecdxrd	273	-----tg-----aggatgttga--
Sldxrd	261	-----agtggctcgctacgg--tgatgc--
Mldxrd	272	-----tg-----gt--tgaggaga
Pmdxrp	291	-----tcct-----ga--agcaga--
Atdxrd	509	-----at-----cc--tgaagc--
Cjdxrd	243	-----ta-----ag--ctttta--
Pfdxrd	451	ataaaagattataaacctataatattg-----tg--tggtga--

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Stdxrds	279	ctg-----gacg-----atgg---c-----tgcg---atc---gtcggc
Padxrd	289	gtg-----gacatggtaatgg---c-----ggcc---atc---gtcggc
Zmdxrd	285	ttg-----gaca-----atgg---c-----agcc---att---atcggt
Sgdxrd	285	ctc-----ggtg-----ctga---a-----cggc---atc---accggt
Nmdxrd	291	cgg-----tgtc-----atgt---g-----cgcc---atc---gtcggg
Ecdxrd	285	tca-----ggtg-----atgg---c-----agcc---att---gttgcc
Sldxrd	282	cga-----gatt-----gtggtcac-----tggc---att---gtcggt
Mldxrd	284	ctgaggctgacg-----ttgt---cctcaatgcg---ctg---gtcggg
Pmdxrp	303	tat-----ggta-----atgg---c-----tgcg---att---gtgggg
Atdxrd	519	tgt-----aacc-----gttg---t-----taccggaata---gtaggt
Cjdxrd	253	ctc-----aa-----tgcg---att---gtaggt
Pfdxrd	486	tga-----aggg-----atga---a-----agaa---atatgtagtagta
Stdxrds	304	agcgcagggctcaagccggtgatgg-----
Padxrd	319	gccgccgggctgccgtcgaccctgg-----
Zmdxrd	310	tgcgccggtctaaaagcgacgcttg-----
Sgdxrd	310	tcgatcggcctggccccgacgctgg-----
Nmdxrd	316	gcggtggggctgccttcgcgctcg-----
Ecdxrd	310	gctgctgggctgttacctacgcttg-----
Sldxrd	310	tgcgctggtctgctacccacgatcg-----
Mldxrd	319	gcattgggtctgcgacccacactgg-----
Pmdxrp	328	gcggcgggattattgcctactttgt-----
Atdxrd	547	tgtgcgggactaaagcctacggttg-----
Cjdxrd	271	tttgaggacttaaaagcacttta-----
Pfdxrd	515	atagtatagataaaaatagttatttggtattgattcttttcaaggattatat
Stdxrds	329	-ccgcgctggaggccggtggcacc-----gtcgcgctcgcaacaa
Padxrd	344	-cggccgtcgaggccggcaagcgc-----gtactgctggccaacaa
Zmdxrd	335	-cagctattcgcaagggcaaaacg-----gtcgctttagcgaataa
Sgdxrd	335	-ccgcgctgcgggcccggcggtg-----ctggtgctggcgaacaa
Nmdxrd	341	-cagcggcgcaaaaaggcaaaacc-----atttatctggcgaacaa
Ecdxrd	335	-ctgcgatccgcgcgggtaaaacc-----atthtctggcgaataa
Sldxrd	335	-ccgcgatcgaagccggcaaggat-----atcgcccttgccaacaa
Mldxrd	344	-ctgcactgcacacgggcgcgcga-----ttggcggtggccaacaa
Pmdxrp	353	-ctgcggtgaaagctggaacacgt-----gtactattagcaataa
Atdxrd	572	-ctgcaattgaagcaggaaaggac-----attgctcttgcaacaa
Cjdxrd	296	-aggctaaagagcttggaacaaac-----atagctttagctaataa
Pfdxrd	565	tctactatgtatgcaattatgaataataaaatagttgcgttagctaataa

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```

Stdxrds 369 ggagtcgctcgtctcggcggtgaggtgatgatgg-cggcgcccg-cg
Padxrd 384 ggaggcgtggtgatgtccggcgctgttcacgc-aggcggt-caa-gc
Zmdxrd 375 ggaatccttagtttcagctggcggttgatgatcg-atgccgtgcgg-ga
Sgdxd 375 ggagtcgctgatcgtcggcggtccgctggtgaagg-cggtg-----gc
Nmdxrd 381 agagacgctggtggtttccggcgctgtttatgg-aaaccgcccgt-gc
Ecdxrd 375 agaactcactggttacctgcggacgtctgtttatggacgccgtaaagcaga
Sldxrd 375 agaaaccctgattgcagcaggcccagtggtcctgc-cactcctgcaa-aa
Mldxrd 384 ggaatcgctggttagctggcggttcgctggtgttg-cgcggcg-c---a
Pmdxrp 393 agaagccttggttaacttgcgggcaattatttattg-atgcagtgcgt-ga
Atdxrd 612 agagacattaatcgaggtggtcctttcgtgcttc-cgcttgccaac-aa
Cjdxrd 336 agaaagtctttagtagctgg-gagttttt-----
Pfdxrd 615 agaattccattgtctcgtcgtggtttcttttaaga-aattattaaat-at

Stdxrds 417 gcat-ggc---gagacgtgctgcccgtcgattcggagcacaatgcggtg
Padxrd 431 gcagcggc---gcggtgctcctgcccagtcgacagcgagcacaacgcgac
Zmdxrd 423 acat-ggc---acgacgttctccccgtcgattccgagcataacgctatt
Sgdxd 417 gcag-ccc---ggccagatcgtgcccgtggactccgagcagcccgctg
Nmdxrd 429 aaac-ggc---gcccagtgctgcccgtcgacagcgaacacaacgcggtt
Ecdxrd 425 gcaa-agc---gcaat--tgttaccggtcgatagcgaacataacgccatt
Sldxrd 423 gcac-ggt---gtcaccattacgcctgccgactccgagcactccgcgac
Mldxrd 429 gcca-ggc---caga---tcgtgcccgtagactcggaacactccgcgctg
Pmdxrp 441 atct-caa---gcacaattgttaccagtagatagtgaaacataatgcgatt
Atdxrd 660 acat-aat---gtaaagattcttccggcagattcagaacattctgccata
Cjdxrd 366 gaaa-ggg---gctaaatttttaccggttgatagtgagc---atgcagct
Pfdxrd 663 tcat-aaaaatgcaaagataatacctggttgattcagaacatagtgctata

Stdxrds 463 ttccag----t-----gc---ct-----cg--at----
Padxrd 478 ttccag----t-----cg---ctgccgcgcaattatgccg--at----
Zmdxrd 469 ttccaa----t-----gc---tt-----c---c----
Sgdxd 463 ttccag---g-----cg---ct-----gg--cc----
Nmdxrd 475 ttccaagt---gc---cg-----cgcat---
Ecdxrd 469 tttcag---a-----g---t-----tt--ac----
Sldxrd 469 tttcag---t-----gc---at-----cc--aa----
Mldxrd 472 gcgcaa---t-----gc---ctgcg-----cg--gt----
Pmdxrp 487 ttccaa---tcccttcgc---ct-----ga--ag----
Atdxrd 706 tttcag---t-----gt---at-----t----
Cjdxrd 409 ttaaaa---t-----ttttact-----cg--aa----
Pfdxrd 712 tttcaa---t-----gt---tt-----ag-ataata

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Stdxrds 478 -----cg-----ca-----ccgc---
Padxrd 508 -----gg-----cc-----tgga---
Zmdxrd 482 -----cg-----catcataa-----ccgc---
Sgdxrd 478 -----gg-----cg-----gcgc---
Nmdxrd 496 -----ta-----ca-----cagg---
Ecdxrd 482 -----cg-----ca-----acct---
Sldxrd 484 -----gggctttca-----acccatg
Mldxrd 490 -----gg-----ta-----cc-----
Pmdxrp 509 -----cg-----caaagacaaattgggttttgccgc---
Atdxrd 718 -----ca-----ag-----gttt---
Cjdxrd 427 -----gg-----ta-----aaaa---
Pfdxrd 731 ataaggtattaaaaaca-----aa-----atgt---

Stdxrds 486 -----gccagg-----ggcg---tccgccg-----ga
Padxrd 516 -----gcgggtc-----ggcg---tgcgccg-----ga
Zmdxrd 496 -----gacta-----tg---ttcgccg-----ga
Sgdxrd 486 -----ccgcgcg-----gagg---tccgcaa-----gc
Nmdxrd 504 -----tcgcctg-----aacg---aacacgg-----ca
Ecdxrd 490 -----atccagcataatct-ggga---tacgtgaccttga
Sldxrd 500 ctgattttcggcctgctcaagtcgtggcagggc---tgcgacg-----ga
Mldxrd 496 -----cccgac-----gaag---ttgctaa-----gt
Pmdxrp 536 -----tttctgaatta---ggga---tcagtaa-----ga
Atdxrd 726 -----gcctgaa-----ggcgctctgcgcaa-----ga
Cjdxrd 435 -----aaatata-----gcaa---aacttta-----ta
Pfdxrd 754 -----ttacaag-----acaa---tttttct-----aa

Stdxrds 506 tc-----a-----tccttacc
Padxrd 536 tc-----c-----tcttgacc
Zmdxrd 512 tt-----a-----ttattacg
Sgdxrd 506 tg-----g-----tggtgacc
Nmdxrd 524 tcgcttcgatt-----a-----tcctgacc
Ecdxrd 522 gc-----aaaatggcggtggtgtccattttacttacc
Sldxrd 542 tt-----c-----tcctgact
Mldxrd 515 ta-----g-----tgctaacc
Pmdxrp 560 tt-----g-----tgттаacg
Atdxrd 749 ta-----a-----tcttgact
Cjdxrd 455 tc-----aca
Pfdxrd 774 aattaacaatataaaataaata-----tttttatg

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Stdxrds	517	gccagc-ggtggtccgttccgcg--cg----acgccgaaggaagcgatgc
Padxrd	547	gcctcc-ggcggcccgttccgcg--ag----acgccgctgga-gcaactc
Zmdxrd	523	gccagc-ggaggtcccttcagaa--ca----acgtctcttgccgaaatg-
Sgdxrd	517	gccagc-ggcggcccgttccgcaaccg----caccgctgagcagc--tgg
Nmdxrd	544	gcttcc-ggcggcccgtttctga--c-----cgccgatttaaac-acgt
Ecdxrd	553	gggtct-ggtggccctttccgtg--ag----acgcc--attgcgcgattt
Sldxrd	553	gccagt-ggcggcgcttttcggg--ac----tggccggctcgaacggctgt
Mldxrd	526	gcctcc-ggcgggcccgtttcgtg--gctggaacgccg-gcgacttgagc
Pmdxrp	571	ggatcc-ggtggtccattccgtt--at----acccctctgga-gcaattt
Atdxrd	760	gcatct-ggtggagcttttaggg--at----tggcctgtcgaagctaa
Cjdxrd	460	gcaagt-ggtggagctttttata--gg----tataaaatcaaagatttaa
Pfdxrd	804	ttcatctggaggtccatttcaaa--at----ttaactatggacgaattaa
Stdxrds	560	gcg-ac--a-tca---ccccgcacaggcggtggcg-catcccaactggt
Padxrd	589	gct-tc--ggtga---cgccggagcaggcttggtgcg-caccgaaactggt
Zmdxrd	565	gca-ac--ggtca---cgccagaacgcgcggttcag-catcccaactggt
Sgdxrd	560	cgg-cc--g-tca---cgccggcgcgacgcgtggcg-caccgacctggg
Nmdxrd	584	tcg-ac--a-gcattacgcccagccaagcggtcaaa-cacccaattggc
Ecdxrd	594	ggc-aacaa-tga---cgccgatcaagc-ctgccgtcatccgaactggt
Sldxrd	596	cgc-aa--g-taa---ctgtcgcagatgcgctcaag-catcccaactggt
Mldxrd	572	gcg-----tta---caccgagcaggcgggcgctc-catccgacttggt
Pmdxrp	613	gaacag--a-tca---cccagcacaagcagttgcg-catcctaattggt
Atdxrd	803	agg-aa--g-tta---aagtagcggatgcgttgaag-catccaaactgga
Cjdxrd	503	atc-aa--g-tca---gtgtcaaagatgctttaaaa-catcctaattgga
Pfdxrd	848	aaa-at--g-taa---catcagaaaatgctttaaag-catcctaattgga
Stdxrds	602	cgatgggcgccaagatctcggtcgactccgcgacgatgatgaacaagggg
Padxrd	632	cgatggggcgtaagatttccgtcgactccgccagcatgatgaacaagggg
Zmdxrd	608	caatgggtgccaagatttctatcgattctgctacaatgatgaataagggg
Sgdxrd	602	cgatgggcccgtggtgacgatcaactcggcgaccctggtgaacaagggc
Nmdxrd	629	gtatgggacgcaaaatctccgtcgattccgccaccatgatgaacaaaggt
Ecdxrd	638	cgatggggcgtaaaatttctgtcgattcggctaccatgatgaacaaaggt
Sldxrd	638	cgatggggcgcaagattaccgtcgactccgccaccttgatgaataaaggc
Mldxrd	611	caatggggacgatgaacacgctgaactcagcgtctctggttaacaagggg
Pmdxrp	656	caatggggaaaaagatctctgtcgattccgctaccatgatgaataaaggg
Atdxrd	845	acatgggaaagaaaatcactgtggactctgctacgcttttcaacaagggg
Cjdxrd	545	acatgggagcaaagatcactatagatagtgcgactatggcaataagctt
Pfdxrd	890	aaatgggtaagaaaataactatagattctgcaactatgatgaataaaggt

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Stdxrds	652	ctcgaactgatcgaagccttccacctgttcccgggtcgcc--gccgagcaa
Padxrd	682	ctcgaactgatcgaggcgtgctggtggtc---gacgcccagccgagcca
Zmdxrd	658	cttgaattgatagaagcctatcatctcttccagattcca--ttagaaaaa
Sgdxrd	652	ctggaggtgatcgaggcgacactgctgtacgacgtgccg--ttcgaccgg
Nmdxrd	679	ttggagctgattgaagcgcatctggctgttcaactgtccg--cccgacaaa
Ecdxrd	688	ctggaatacattgaagcgctgtgctgtttaacgccagc--gccagccag
Sldxrd	688	ctcgaggtgatcgaagcccactatctcttcggcttggat--tacgactac
Mldxrd	661	ctcgagctcatcgaagccaacctgtgttcggcattccc--tacgaccgc
Pmdxrp	706	ttggaatatattgaagcacgctgggtattttaatgcctcg--gcagaagaa
Atdxrd	895	cttgaggtcattgaagcgcatctattgtttggagctgag--tatgacgat
Cjdxrd	595	tttgagattatagaggcttatcatttat-----atgat--tttaaagaa
Pfdxrd	940	ttagaggttatagaaacccattttttatttgatgtagat--tataatgat
Stdxrds	700	c-tggccgtgctgggtccatcgccaatccgtcgtccattcgatggtggaat
Padxrd	729	ggtcgaggtggtgatccaccgcgagcgatccactcgatggtggact
Zmdxrd	706	t-ttgaatttttggttcacctcagtcagttattcactccatggtggaat
Sgdxrd	700	a-tcgaggtggtgggtccatccgcagtcggtcggttcattcgatggtggaat
Nmdxrd	727	c-tcgaagtgcgtcatccatccgcaatctgtgatacacagcatggtgcgct
Ecdxrd	736	a-tggaagtgcgtgattcaccgcgagtcagtgattcactcaatggtgcgct
Sldxrd	736	a-tcgacatcgatccatcccgagcatccactcgctgatttgagc
Mldxrd	709	a-ttgaggtggttggtgcaccctcagtcgaattgttcattcgatggtgacat
Pmdxrp	754	a-tggaagtattattcatcctcaatccattattcattctatggtacgtt
Atdxrd	943	a-tagagattgtcattcatccgcaaagtatcatatccatgattgaaa
Cjdxrd	637	a-ttgatgctttaatagaaccaagatctttagtgcagtgcaatggtggaat
Pfdxrd	988	a-tagaagttatagtacataaagaatgcattatacattcttgtgtggaat
Stdxrds	749	atgtcgacggatcggtgctggcccagctcggcacgcccagacatgcgcacg
Padxrd	779	acgtcgacggttcggtgatcgcccagctcggaatccggacatgcgcacg
Zmdxrd	755	atttgatggttctatccttgcccagatcggtagtcctgatatgagaaca
Sgdxrd	749	tcgtggacggttcgacgatggcccaggccagcccgcggacatgcgcacg
Nmdxrd	776	accgcgacggtccgtggttgccgcaactgggcaatcccgatatgcgaacg
Ecdxrd	785	atcaggacggcagtggtctggcgagctgggggaaccggatatgcgtacg
Sldxrd	785	tagaagatacctccgtcttggcgcaattgggctggccggatatgcgactg
Mldxrd	758	tcatcgacggctcgacgatcgccaagccagccctccggacatgaagcta
Pmdxrp	803	acatcgatgggtccgtgattgctcaaatggggaatcctgatatgcgtaca
Atdxrd	992	cacaggattcatctgtgcttgctcaattgggttggtgatatgcgttta
Cjdxrd	686	ttaaaaatggagctagcacggcgtatttttcaaaagcagatatgaaacta
Pfdxrd	1037	ttatagacaaatcagtaataagtcaaatgtattatccagatatgcaata

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```

Stdxrds 799 ccgatcgccctatgcgctggcttggcccagcgga----t-----g
Padxrd 829 ccgatttcctatgcatggcctggccggagcgaa----t-----c
Zmdxrd 805 ccgatcggtcatactttggcttggccaaagcgga----t-----g
Sgdxd 799 ccgatcgcgctgggcctcggttggccggaccggg----t-----g
Nmdxrd 826 cctatcgcttattgtttgggttggccgagcgca----t-----c
Ecdxrd 835 ccaattgcccacacatggcatggccgaatcgcg----t-----g
Sldxrd 835 cccttgctctacgccctctcctggcccgatcgcc----t-----c
Mldxrd 808 cctatttctttggcgcttgggttggccacagcggg----t-----g
Pmdxrp 853 ccgattgcggaaccatggcatatccaagtcggaccgtt-----g
Atdxrd 1042 ccgattctctacacatgtcatggccgatagag----ttccttggtctg
Cjdxrd 736 gctatttcagatgctatattt-----gaaaaac-----a-----a
Pfdxrd 1087 cccatattatattctttaacatggcctgatagaa----t-----a

```

```

Stdxrds 835 gag-acgc---tgtgccc---gccgc-t-cgaccttg-----ccac
Padxrd 865 gat-tccg---gcgtttc---gccgc-t-ggatatgt-----tcgc
Zmdxrd 841 gaa-acac---cagccga---atcgt-t-ggatttta-----ccaa
Sgdxd 835 ccggacgc-----cgccc---ccggc-tgcgactgga-----ccaa
Nmdxrd 862 gat-tcgg---gtgtcg---gcgacct-ggatttcg-----acgc
Ecdxrd 871 aa-----c---tctggcgtgaagccgc-t-cgattttt-----gcaa
Sldxrd 871 tct-actc---aatggtc---ggcgc-t-cgatctgg-----tcaa
Mldxrd 844 g-----gtg-gc---gctgc-t-cgagcctgtgctttcactac
Pmdxrp 893 ctg-gcgt---tgagccc-----t-t-ggattttt-----acca
Atdxrd 1088 aag-taac---t-tggcc---aagac-t-tgaccttt-----gcaa
Cjdxrd 766 gat-acgcctattttaga---ggctg-t-tgatttta-----gca-
Pfdxrd 1123 aaa-acaa---attttaa---acctt-t-agatttgg-----ctca

```

```

Stdxrds 867 ggtgggtaagctcgagttcgaaaatcccgatctcgatcgcttc-----
Padxrd 897 cgtcggtcgccctggatttccagcgccccgacgagcagcgcttc-----
Zmdxrd 873 attgcgccagatggattttgaagcaccagattatgaacgtttt-----
Sgdxd 867 ggccgcgacctgggagttcttcccgcgtggacaacgaggcgcttc-----
Nmdxrd 894 attgtccgcgctgaccttccaaaagcccgactttgaccgcttc-----
Ecdxrd 903 actaagtgcgttgacatttggcgcaccggattatgatcggttat-----
Sldxrd 903 agcgggcagcttggagttccgggaaccggatcacgccaatac-----
Mldxrd 876 cgcattctacctgggaattcgagccgctggacatcgatgtttt-----
Pmdxrp 921 actgaatggattaacctttattgagccagactatcaacgttat-----
Atdxrd 1119 actcggttcattgactttcaagaaaccagacaatgtgaaatac-----
Cjdxrd 800 -----aaatgcctgctttaaaatttc-atc-caatcagcacaacaaaaa
Pfdxrd 1155 ggtttcaactcttacatttcataaaccttctttagaacatttc-----

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```

Stdxrds  910 ---ccggcgctcgcgctggcgatggaggcattgaag-gcgggcggggcgc
Padxrd    940 ---ccctgcctgcgcctggcgagccaggccgcggaa-accggcggcagcg
Zmdxrd    916 ---ccggcattaactttggcaatggaatccatcaaa-tcagggtggggctc
Sgdxrd    910 ---ccggcggtcgagctggcccgcgaggtgggtacg-ctcggcgggaccg
Nmdxrd    937 ---ccctgcctgaagctcgccatgaagccatgaac-gcaggcgggagccg
Ecdxrd    946 ---ccatgcctgaaactggcgatggaggcggttcgaa-caaggccaggcag
Sldxrd    946 ---ccctgcatggacttggcctacgcgcgggtcgc-aaaggcggcacaa
Mldxrd    919 ---cccgcagtcgagctggccggcacgctggacag-atcggcggtgta
Pmdxrp    964 ---ccttgtttaaaattagctattgacgcattttca-gccggacaatatg
Atdxrd    1162 ---ccatccatggatccttgcttatgctgc-tggacgagctggaggcacia
Cjdxrd    841 tatictattttaagcttaaaaatacatttttaaaa-gagccaaatttag
Pfdxrd    1198 ---ccgtgtattaaattagcttatcaagcaggtata-aaaggaaactttt

Stdxrds  956 gtccggccattctcaatgccgccaacgaagtcgccgtcgggcctttctc
Padxrd    986 ccccggccatgctgaatgccgcgaacgaggtggccgtggccgcattttctc
Zmdxrd    962 gtccgtgctgtaataatgccgctaataaataagcttgggcgcccttcctt
Sgdxrd    956 ccccgcggtcttcaatgccgccaacgaggaatgtgtggacg-ctttcct
Nmdxrd    983 cgccctgcgtattgaacgccgccaacgaagccgccgtcgccgcctttttg
Ecdxrd    992 cgacgacagcattgaatgccgcaaacgaaatcaccgttgctgcttttctt
Sldxrd    992 tgccagccgtcttgaatgccgcaaatgagcaagccgtcgccctcttccta
Mldxrd    965 tgaccgccatttacgatgctgctaataaggaggctgcagaggccttcctc
Pmdxrp    1010 ccacgacagcaatgaatgcagcgaatgaaatcgcggtagcgtctttctta
Atdxrd    1208 tgactggagttctcagcgccgccaatgagaaagctgttgaaatgttcatt
Cjdxrd    890 gt---gttatcatcaatgctgctaataagttggtgtttataatttttta
Pfdxrd    1244 atccaactgtactaaatgctgtaaatgaaatagctaacaacttatttttg

Stdxrds  1006 gccgggcggtat-----c-----ggattccttgaaa-ttgccg
Padxrd    1036 gagcggcacat-----c-----cgcttcagcgaca-tcgcg
Zmdxrd    1012 gataagaaaat-----c-----ggttttcttgata-tcgcta
Sgdxrd    1005 gaaggcgcgactgcccttcacc-----ggaatcgtggaaca-ctgtgg
Nmdxrd    1033 gacggacagat-----t-----aagtttaccgaca-ttgcca
Ecdxrd    1042 gcgcaacaaat-----c-----cgctttacggata-tcgctg
Sldxrd    1042 gaggagcaaat-----t-----cacttctcggata-ttcgcg
Mldxrd    1015 caaggtcggat-----c-----ggcttccccgcca-tcgctg
Pmdxrp    1060 gacaataagat-----t-----aaattcacagata-ttg---
Atdxrd    1258 gatgaaaagat-----aagctatttgatattctcaagggtgtg
Cjdxrd    937 gaaaataaaaag-----t-----ggatttttagaca-ttgcta
Pfdxrd    1294 aataataaaaat-----t-----aaatattttgata-tttcct

```

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Stdxrds 1037 c----aatctctgccg----atagctgtctcgctatgac----ccgg--
 Padxrd 1067 t----tatcatcgagg----acgtgctgaaccgagggcg----gtga--
 Zmdxrd 1043 a----aattgtcgaga----aaacattagatcattataca----cccg--
 Sgdxrd 1046 c----gaaggtggtcgccgaacacggcacaccgcaat-----cgg--
 Nmdxrd 1064 a----aaccgtcgccc----attgtctttcac---aagacttttcaga--
 Ecdxrd 1073 cgttgaaatttatccgt----a----ctggaaaaaatggat----atgc--
 Sldxrd 1073 g----cctgattgaac----gtgcctgcgatcgccaccaa----acgg--
 Mldxrd 1046 c----aacaatcgcgg----atgtgttgagcggtgccgac----caat--
 Pmdxrp 1088 -----cgcgacta----aatcagttagtcgtgagcaa----attg--
 Atdxrd 1298 a----attaacatgcg----ataaac---atcgaaacgag---ttggta
 Cjdxrd 968 a----atgcattttta----aagcccttgatcattttgga----gtac--
 Pfdxrd 1325 ctat-aatatcgcaag----ttcttgaatctttcaattct----caaa--

Stdxrds 1073 -ccgcgcc---g-----gaaacgc-----tc---g-----atg---
 Padxrd 1103 -ccgcagt---c-----gaatcgc-----tc---g-----atc---
 Zmdxrd 1079 -caacccc---g-----tcttctt-----tg---g-----aag---
 Sgdxrd 1082 -gaacttc---g-----ctcacgg-----tg---g-----agg---
 Nmdxrd 1101 -cggcata---g-----gcgac-a-----ta---g-----ggg---
 Ecdxrd 1109 -gcgaacc---a-----caatgtg-----tg---g-----acg---
 Sldxrd 1109 -agtggcaacag-----caaccga-----gcttgg-----atg---
 Mldxrd 1082 -gggctcc---c-----caatggg-----gt---g-----agggac
 Pmdxrp 1120 -caaccac---a-----aaaaattcattgcata---g-----aag---
 Atdxrd 1333 acatcacc---gtctcttgaagaga-----tt---gttcactatg---
 Cjdxrd 1004 -ctaaaat---t-----tcaagca-----ta---g-----aag---
 Pfdxrd 1364 -aggtttc---g-----gaaaata-----gt---g-----aag---

Stdxrds 1094 ccg----tgctggc---g-----atcga-----cgc--gga
 Padxrd 1124 agg----tctggctgccg-----atcgc-----cgc-----
 Zmdxrd 1100 atg----tctttgc---g-----atcga-----caa--tga
 Sgdxrd 1103 acg----tac-----tcca-----cgc--gga
 Nmdxrd 1121 ggc----tcttggc---g-----caaga-----tgcccgga
 Ecdxrd 1130 atg----tggtatc---t-----gttga-----tgc--gaa
 Sldxrd 1136 aca----ttttggc---c-----tacga-----cgc--ttg
 Mldxrd 1106 ccgctactgtggat---g-----atgta-----ctc--ga
 Pmdxrp 1148 atg----tacttga---g-----gtaga-----taa--aaa
 Atdxrd 1367 act----tgtgggc---a-----cgtgaatatgccgc--gaa
 Cjdxrd 1025 aag----tttttga---g-----tatga-----
 Pfdxrd 1385 att----taatgaa---gcaaattctacaaatata-----ttc--ttg

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```

Stdxrds 1116 g-gcgcggc--tttacgcggctgagcg-agtg-----
Padxrd 1147 --gcgcg----ttcggtcgccgggca-atgg-----
Zmdxrd 1122 a-gcgcgga--tacaagccgctgcttt-aatg-----
Sgdxrd 1119 gagctgggc-----ccgggccggggcc-cgcg-----
Nmdxrd 1145 c-acgcgca--caagcgcg---gca-ttta-----
Ecdxrd 1152 c-gcgcg----tgaagtcgccaga---aaag-----
Sldxrd 1158 g-gcacggcagtttgcaagctagct-atca-----
Mldxrd 1131 c-gcgcagc--gctgggcccgtgagcg-agcgttggtgcggtagcaaca
Pmdxrp 1170 g-gcaagggaattatctcagtcfaatca-tttt-----
Atdxrd 1395 t-gtgcagc--tttcttctg--gtgct-aggc-----
Cjdxrd 1041 -----ttttaaaacaagagagtattt-----
Pfdxrd 1419 g-gccaaag--ataaagctaccgatat-atac-----

Stdxrds 1144 -----aag---gactgc--gtcg---cttga-----
Padxrd 1171 -----ttg---acccgg--cacg---ccggctag-----
Zmdxrd 1150 -----gag---agtttg--cccg---cgtga-----
Sgdxrd 1145 -----a-----gctggcgggccg---gctga-----
Nmdxrd 1169 -----tcg---gcacac--tgcg---c-tga-----
Ecdxrd 1175 -----agg---tgatgc--gtct---cgcaagctga-----
Sldxrd 1188 -----aagtctggaatcc--gtcg---tttag-----
Mldxrd 1177 gcgagttctggaaag---gtctct--gacatggtcttagaaaggccta
Pmdxrp 1200 -----aag---tttttc--acat---ccgtaa-----
Atdxrd 1421 -----cag-----ttc--at-g---catga-----
Cjdxrd 1062 -----aag---ga-----gttaa-----
Pfdxrd 1447 -----aac---aaacat--aatt---cttcatag-----

Stdxrds 1162 -
Padxrd 1192 -
Zmdxrd 1168 -
Sgdxrd 1162 -
Nmdxrd 1186 -
Ecdxrd 1198 -
Sldxrd 1210 -
Mldxrd 1221 a
Pmdxrp 1219 -
Atdxrd 1435 -
Cjdxrd 1072 -
Pfdxrd 1468 -

```

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Stdxrp	1	-----
Zmdxrp	1	ms-----
Padxrp	1	ms-----
Ecdxrp	1	-----
Nmdxrp	1	m-----
Hidxrp	1	m-----
Ssdxrp	1	-----
Pmdxrp	1	msisy-----
Sldxrp	1	-----
Sgdxrp	1	-----
Bsdxrp	1	-----
Mldxrp	1	mnn-----
Mtdxrp	1	matggrv-----
Atdxrp	1	mmtlnslspaeskaisfldtsrfnpipklsggfsllrrrnqgrgfgkgvkc
Cjdxrp	1	-----
Pfdxrp	1	mkkyiyiyffffititindlvinnntskcvsierrknnayinygigygngpdn
Stdxrp	1	-----vvk-----r-----
Zmdxrp	3	-----qpr-----t-----
Padxrp	3	-----rpq-----r-----
Ecdxrp	1	-----mk-----q-----
Nmdxrp	2	-----tpq-----v-----
Hidxrp	2	-----qkq-----n-----
Ssdxrp	1	-----mvk-----r-----
Pmdxrp	6	-----fmk-----k-----
Sldxrp	1	-----mk-----a-----
Sgdxrp	1	-----
Bsdxrp	1	-----mk-----n-----
Mldxrp	4	-----pieghaggrlr-----
Mtdxrp	8	-----vir-----rrgdnevvahnd
Atdxrp	51	svkvqqqqpppawpggravpeaprqswdgpk-----p-----
Cjdxrp	1	-----
Pfdxrp	51	kitksrrckrikclckdlidig-----aik-----kpin-----

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Stdxrp	5	-----vtvlgatgsvgtstldlie---rnphafevvalta
Zmdxrp	7	-----vtvlgatgsighstldlie---rnl dryqviaalta
Padxrp	7	-----isvlgatgsigl stldvvq---rhp dryeafaltg
Ecdxrp	4	-----ltilgstgsigcstldvvr---hnpehfrvvalva
Nmdxrp	6	-----ltilgstgsigestldvvs---rhpekfrvfalag
Hidxrp	6	-----ivilgstgsigkstlsvie---nnpqkyhafalvg
Ssdxrp	5	-----isilgstgsigtqtldivt---hhpdafqvvglaa
Pmdxrp	10	-----ivilgstgsigtstlsvit---hnpdkyqvvalvg
Sldxrp	4	-----vtllgstgsigtqtldile---qypdrfrlvglaa
Sgdxrp	1	-----mvilgstgsigtqaidvvl---rnpgrfkvvalsa
Bsdxrp	4	-----icllgatgsigeqtldvlr---ahqdqqlvsmsf
Mldxrp	15	-----vlvgstgsigtqalevia---anpdrfevvglaa
Mtdxrp	23	evtnstdgradgrlrvvlgstgsigtqalqvia---dnpdrfevvglaa
Atdxrp	83	-----isivgstgsigtqtldiva---enpdkfrvvalaa
Cjdxrp	1	-----milfgstgsigvnalklaa---lk--nipsisalac
Pfdxrp	80	-----vaifgstgsigtalniirecnkienvfnvkalyv
Stdxrp	37	-n-cdveklaaaairtrarcavvadekclpalqerla--g---s---g
Zmdxrp	39	-n-rnvkd ladaakrtnakraviadpslyndlkeala--g---s---s
Padxrp	39	-f-srlaelealclhrpvyavvpeqaaaialqgsia--a---a---g
Ecdxrp	36	-g-knvtrmveqclefspryavmddeasakllktmlqqgg---s---r
Nmdxrp	38	-h-kqveklaaqcqtfhpeyavvadaehaarleallkrdg---t---a
Hidxrp	38	-g-knveamfeqcikfrphfaalddvnaakilrekli--a---h---h
Ssdxrp	37	-g-gnvallaqqvae frpeivairqaekledlkaava--el---t---d
Pmdxrp	42	-g-rnvelmfqqcltfqpsfaaldddvaakmlaeklk--ahq--s---q
Sldxrp	36	-g-rnvallseqirrrhrpeivaiqdaaqlselqaala--dld--n---p
Sgdxrp	33	ag-gavellaeqavalgvhtvavad---paaeeaaar-g---p---g
Bsdxrp	36	-g-rnidkavpmievfqpkfsvsgdldtyhklkqmsf--s---f---e
Mldxrp	47	-ggaqldtllrgraatgvtniaiaddra-----aqla--g---dipyhg
Mtdxrp	70	-ggahldtllrgraqtgvtniavadehaaqrvgd-----
Atdxrp	115	-g-snv tlladqvr rfkpalvavrneslinelkeala--d---l---d
Cjdxrp	31	-g-dniallneqiarfkpkfvsikdsknkhlvkhdrv--f---i---g
Pfdxrp	115	-n-ksvnelyeqareflpeylcihdksvyeelkelvk--nikdyk---p

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Stdxrp	75	--v--ea--mg-----gahsvcdva-rm-g-adwtmaa-ivgsaglk
Zmdxrp	77	--v--ea--aa-----gadalveaa-mm-g-adwtmaa-iigcaglk
Padxrp	77	--i--rtrvlf-----gegalceva-sa-pevdmvmaa-ivgaaglp
Ecdxrp	76	--t--ev--ls-----gqqaacdma-aled-vdqvmaa-ivgaagll
Nmdxrp	78	--t--qv--lh-----gaqalvdva-sa-devsgvmca-ivgavglp
Hidxrp	76	--iptev--la-----grraiceaahp-d-adqimas-ivgaagll
Ssdxrp	76	--y--qp--myvv-----geegvveva-ry-gdaesvvtg-ivgcagll
Pmdxrp	82	--t--tv--la-----gqqaiceaahp-e-admvmaa-ivgaagll
Sldxrp	76	--p--li--lt-----geagvteva-ry-gdaeivvtg-ivgcagll
Sgdxrp	69	--g--qg--agrplprvlagpdaatela-aa-e-chsvlng-itgsigla
Bsdxrp	74	--c--qi--gl-----geeglieaa-vm-eevdivvna-llgsvgli
Mldxrp	85	--t--da--vt-----rl-----ve-et-e-advvlna-lvgalglr
Mtdxrp	103	--i--py--hg-----sdaatrlve-qt-e-advvlna-lvgalglr
Atdxrp	153	ykl--ei--ip-----gegqvieva-rh-p-eavtvvtg-ivgcaglk
Cjdxrp	69	--q--eg--le-----qiltecqdk-ll-----lna-ivgfaglk
Pfdxrp	157	--i--il--cgde-----gmkeic--s-sn-s-idkivig-idsfqgly
Stdxrp	107	pvmaleaggtvalankeslvvsagevmmaarah-gatltpvdsehnaf
Zmdxrp	109	atlaairkgktvalankeslvvsagglmidavreh-gttltpvdsehnaf
Padxrp	112	stlaaveagkrvllankealvmsgalvmqavkrs-gavllpidsehnaf
Ecdxrp	109	ptlaairagktillankeslvvtcgrlfmdavkqs-kaqltpvdsehnaf
Nmdxrp	111	salaaaqkgktilanketlvsvgalmetaran-gaavlpvdsehnaf
Hidxrp	111	ptlsavkagkrvllankeslvvtcgqlfidavkny-gskllpvdsehnaf
Ssdxrp	111	ptmaaiagkdialanketliagapvvplvekm-gvklpadsehsaif
Pmdxrp	115	ptlsavkagkrvllankealvtcgqlfidavres-qaqltpvdsehnaf
Sldxrp	109	ptiaaieagkdialanketliaagpvplllqkh-gvtitpadsehsaif
Sgdxrp	109	ptlaalragrvlvlankealivggplvkavaqp---gqivpvdsehaalf
Bsdxrp	107	ptlkaieqkktialanketlvtaghivkehakky-dvpllpvdsehsaif
Mldxrp	112	ptlaalhtgarlalankeslvaggsvlaaaqp---gqivpvdsehsala
Mtdxrp	135	ptlaalktgarlalankeslvaggsvlraarp---gqivpvdsehsala
Atdxrp	188	ptvaaieagkdialanketliagppfvlplankh-nvkilpadsehsaif
Cjdxrp	96	stlkakelgknialankeslvvagsfl-----k-gakflpvdsehaalk
Pfdxrp	189	stmyaimnnkivalankesivagfflklklnihknakiipvdsehsaif

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Stdxrp	156	qcldrtap-----r-----g-----vrriiiltasgpp
Zmdxrp	158	qcfphhnr-----d-----y-----vrriiiltasgpp
Padxrp	161	qslprnya-----d-----glervgvrriiiltasgpp
Ecdxrp	158	qslpqpiq-----hnlgyadleqng-----vvsilltgsaggp
Nmdxrp	160	qvlprdytgrlne-----h-----g-----iasiiiltasgpp
Hidxrp	160	qslppeaq-----ekigfcplselg-----vskiiltgsaggp
Ssdxrp	160	qclqgvpe-----g-----g-----lrriiiltasgga
Pmdxrp	164	qslppeaq-----rqigfcplselg-----iskivltgsaggp
Sldxrp	158	qciqglst-----hadfrpaqvvg-----lrriiiltasgga
Sgdxrp	156	qalaggar-----a-----e-----vrklvvtasgpp
Bsdxrp	156	qalqgeqa-----k-----n-----ierliiltasggs
Mldxrp	159	qclrggtp-----d-----e-----vaklvltasgpp
Mtdxrp	182	qclrggtp-----d-----e-----vaklvltasgpp
Atdxrp	237	qciqglpe-----g-----a-----lrkiiltasgga
Cjdxrp	139	flle--gk-----k-----n-----iaklyitasgga
Pfdxrp	239	qcldnnkvltkclqdnfskin-----n-----inkiflcssaggp
Stdxrp	178	fratpkeamrditpaqavahpnwsmgakisvdsatmmnkglelieafhlf
Zmdxrp	180	frrtslaematvtperavqhpwnwsmgakisidsatmmnkglelieayhlf
Padxrp	188	fretpleqlasvtpeqacahpnwsmgrkisvdsasmmnkglelieacwlf
Ecdxrp	190	fretplrdlatmtpdqacrhpwnwsmgrkisvdsatmmnkgleliearwlf
Nmdxrp	187	fltadlntfdfsitpdqavkhpwnwsmgrkisvdsatmmnkglelieahwlf
Hidxrp	192	frytpleqftnitpeqavahpnwsmgkksvdsatmmnkgleliearwlf
Ssdxrp	182	frdlpverlpfvtvqdkhpnwsmgqkitidsatlmnkglevieahylf
Pmdxrp	196	frytpleqfeqitpaqavahpnwsmgkksvdsatmmnkgleliearwlf
Sldxrp	190	frdwpverlsqvtvadalkhpnwsmgrkitvdsatlmnkglevieahylf
Sgdxrp	178	frnrtreglaavtpadalahptwamgpvvtinsatlvnkglevieahllf
Bsdxrp	178	frdktreelesvtvedalkhpnwsmgakitidsatmmnkglevieahwlf
Mldxrp	181	frgwnagdlervtpeqagvhtwsmgmtmntlnsaslvnkglelieanllf
Mtdxrp	204	frgwsaadlehvtpeqagahptwsmgpmntlnsaslvnkgleviethllf
Atdxrp	259	frdwpveklkevkvadalkhpnwnmgkkitvdsatlfnkglevieahylf
Cjdxrp	159	fyrykikdlngvsvkdalkhpnwnmgakitidsatmanklfeieayhly
Pfdxrp	274	fqnltmdelknvtsenalkhpkwkmgkkitidsatmmnkgleviethflf

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Stdxrp	228	pvaaeqlavlvhrqsvvhsmveyvdgsvlaqlgtpdmrtpiayalawper
Zmdxrp	230	qiplekfeilvhppqsvihsmveyldgsilaqigspdmrtpightlawpkr
Padxrp	238	daqpsqvevvihpqsvihsmvdyvdgsviaqlgnpdmrtpisyamawper
Ecdxrp	240	nasasqmevlihpqsvihsmvryqdgsvlaqlgepdmrtpiahtmaawnr
Nmdxrp	237	ncppdklevvihpqsvihsmvryrdgsvlaqlgnpdmrtpiayclglper
Hidxrp	242	nasaeemeviihpqsvihsmvryvdgsvitqmgnpdmrtpiaetmayphr
Ssdxrp	232	glidydhidivihpqsiihslievqdtsvlaqlgwpdmrlpillyalswper
Pmdxrp	246	nasaeemeviihpqsvihsmvryidgsviaqmgnpdmrtpiaetmaypsr
Sldxrp	240	glidydyidivihpqsiihslieleddtsvlaqlgwpdmrlpillyalswper
Sgdxrp	228	dvpfdrievvvhpqsvvhsmvefvdgstmaqasppdmrmpialglgwpdr
Bsdxrp	228	dipyeqidvvlhkesiihsmvefhdksviaqlgtpdmrvpiqyaltypdr
Mldxrp	231	gipydrievvvhpqsvihsmvtfidgstiaqasppdmklpislalagwpqr
Mtdxrp	254	gipydridvvhppqsvihsmvtfidgstiaqasppdmklpislalagwpr
Atdxrp	309	gaeyddieivihpqsiihsmietqdssvlaqlgwpdmrlpilytmswpdr
Cjdxrp	209	df--keidalieprslvhamcefknghostayfskadmklaisdaif--ek
Pfdxrp	324	dvdyndievivhkeciihscvefidksvisqmyypdmqipilysltwper
Stdxrp	278	m---et-l-cppldlatvgklefenpdldrfpalalamealkaggarpai
Zmdxrp	280	m---et-p-aesldftklrqmdfeapdyerfpaltlamesiksggarpav
Padxrp	288	i---ds-g-vspldmfavgrldfqrpdqrfrpclrslasqaetggsapam
Ecdxrp	290	v---ns-g-vkpldfcklsaltfaapdydrypcclklameafeqqgaatta
Nmdxrp	287	i---ds-g-vgdldfdalsaltfqpdpdrfpclklameamaggaapcv
Hidxrp	292	t---fa-g-vepldfffkikeltfiepdrfnrpnklklaidafaagqyatta
Ssdxrp	282	i---yt-d-wepldlvkagslsfrepdhdkypcmqlaygagraggampav
Pmdxrp	296	t---va-g-vepldfyqlngltfiepdyqrypcklklaidafagqyatta
Sldxrp	290	l---st-q-wsaldlvkagslefrepdhakypcmdlayaagrkggtmpav
Sgdxrp	278	v---pd-a-apgcdwtkaatweffpldneafpavelarevgtlggtapav
Bsdxrp	278	l---pl-pdakrlelweigslhfekadfrfclqfafesgkiggtmptv
Mldxrp	281	v---gg-a-aracafttastwefepldidvfpavelarhagqiggcmtai
Mtdxrp	304	v---sg-a-aaacdfhtasswefepldtdvfpavelarqagvaggcmtav
Atdxrp	359	vpcsev-t-wprldlcklgsltfkkpdrnvkypsmdlayaagraggtmtgv
Cjdxrp	255	q---dtpi-leavdfskmpalkfhpietkypifklkntflkepnl-gvi
Pfdxrp	374	i---kt-n-lkpldlaqvstltfhkpslehfpcklayqagikgnfyptv

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Stdxrp	323	lnaanevavaaflagrigfleiaaisadtlsry---d---pa-a--pe--
Zmdxrp	325	mnaaneiaavaafldkkigfldiakivektldhy---t---pa-t--ps--
Padxrp	333	lnaanevavaaflerhirfsdiaviiedvlnre---a---vt-a--ve--
Ecdxrp	335	lnaaneitvaaflagqirftdiaalnlsvlekm---d---mr-e--pq--
Nmdxrp	332	lnaaneaavaafldgqikftdiaktvahclsqd---f---sd-g--ig--
Hidxrp	337	mnaaneiaavqafldrqi gfm diakinsktieri---s---py-t--iq--
Ssdxrp	327	lnaaneqavalflqekisfldiprliectcdlyvgqn---ta-s--pd--
Pmdxrp	341	mnaaneiavasfldnkikftdiarl nqlvvs kl---q---pq-k--ih--
Sldxrp	335	lnaaneqavalfleeqihfsdiprlieracdrh---q---te-w--qqqp
Sgdxrp	323	fnaaneecvdaflkgalpftgivdtvakvvaeh---gt--pq-s--gt--
Bsdxrp	324	lnaanevavaaflagkipflaiedciekaltrh---qlk kp-s--wr--
Mldxrp	326	ydaaneaeaaeflqgrigfpaivatiadvlqra---d---qw-a--pq--
Mtdxrp	349	ynaaneaeaaaflagrigfpaivgiadvlhaa---d---qw-avepa--
Atdxrp	407	lsaane kavemfidekis yldifkvvel tcdkhrn-e---lv-t--sp--
Cjdxrp	300	inaanevgvynflenks gfldiak cifkaldhf---g---vp-k--is--
Pfdxrp	419	lnasneiannlflnnkikyfdiss iisqvlesf---n---sqkv--se--
Stdxrp	362	tldavla id--aearlyaaervkdcva-----
Zmdxrp	364	sledvfa id--neariqaaalmeslpa-----
Padxrp	372	sldqvla id--rrarsvagqwltrhag-----
Ecdxrp	374	cvddvlsvd--anarevarkev mrlas-----
Nmdxrp	371	diggllaq d--artraqarafigtlr-----
Hidxrp	376	niddvleid--aqareiaktllre-----
Ssdxrp	369	-letilaad--qwarrtvlen-sacvatrp-----
Pmdxrp	380	ciedvlevd--kkarelsqsiilsfshp-----
Sldxrp	376	slddilayd--awarqfvqasyqslesvv-----
Sgdxrp	363	sltvedvlh--aes--warararelaag-----
Bsdxrp	366	tfkkwtkip--gdtsiqyshkvv-cs-----
Mldxrp	365	wgegp atvddvldaqrwareralcavatassgkv sdmvlers-----
Mtdxrp	390	tvddvl-----daqrwareraqravsgmasvaiastakpgaagrhas t1
Atdxrp	448	sleeivhyd--lwareyaanvqlssgarpvha-----
Cjdxrp	339	sieevfeyd--fktreylrs-----
Pfdxrp	459	nsedlmkqi--lqihswakdkatdiynkhn-----

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Stdxrp	387	---
Zmdxrp	389	---
Padxrp	397	---
Ecdxrp	399	---
Nmdxrp	395	---
Hidxrp	398	---
Ssdxrp	395	---
Pmdxrp	406	---
Sldxrp	403	---
Sgdxrp	387	---
Bsdxrp	389	---
Mldxrp	407	---
Mtdxrp	434	ers
Atdxrp	478	---
Cjdxrp	357	---
Pfdxrp	487	---